NATURE

No. 4638 SATURDAY, SEPTEMBER 20, 1958 Vol. 182

CONTENTS

	Page
Policy, Purpose and Partnership in Education	749
Chemicals and Cancer. By Dr. C. Auerbach, F.R.S.	750
Naphthalene. By Dr. G. Baddeley	751
Colloids, Old and New. By Dr. C. H. Bamford	751
Virus and Vegetable. By Dr. J. H. Hitchborn	752
An Important Herbarium. By Dr. W. B. Turrill, F.R.S.	752
The *Siboga* Polyzoa	753
Geological Notions of Bernard Palissy. By Dr. F. J. North	754
Quantum Mechanics. By Prof. N. Kemmer, F.R.S.	755
Compressible Flow in High-Speed Aerodynamics. By Dr. W. P. Jones	755
Nuclear Theory. By Dr. A. M. Lane	756
Transistors. By D. D. Jones	756
Detachment Relinquished. By F. I. G. Rawlins	757
Rotation of Artificial Earth Satellites. By Prof. R. N. Bracewell and O. K. Garriott	760
Size and Shape Changes of Polyelectrolytes : Conversion of Chemical into Mechanical Energy	762
Architecture of a Protein Molecule. By Dr. J. C. Kendrew	764
News and Views	768
High Polymers. By Prof. C. E. H. Bawn, C.B.E., F.R.S.	772
Comparative Endocrinology. By Drs P. Eckstein	774
Glasgow University Expedition to North Rona and Sula Sgeir. By T. B. Bagenal and H. T. Powell	775
The Institute of Physics. By S. Weintroub	776
Council for the Preservation of Rural England	777
Collected Papers of the Rowett Research Institute	778
Ribonucleoprotein Particles from *Escherichia coli*. By Dr. A. Tissières and Dr. J. D. Watson	778
Follicle Arrangement in Skin from Wild Sheep, Primitive Domestic Sheep and in Parchment. By Dr. M. L. Ryder	781
Salt Glands in Marine Reptiles. By Knut Schmidt-Nielsen and Ragnär Fange	783
Infra-Red Spectra of a Coalification Series from Cellulose and Lignin to Anthracite. By Prof. C. R. Kinney and E. I. Doucette	785

Letters to the Editors :

Undergraduate Academic Record of Fellows of the Royal Society.—C. Gross and L. Hudson	787
Effect of Air Drag on the Orbit of an Earth Satellite.—D. G. Parkyn ; D. G. King-Hele and Dr. D. C. M. Leslie	787
The Fundamental Molecular Event in Muscular Contraction.—Dr. F. G. E. Pautard	788
Calibration of a Calorimeter : a Minor Peaceful Use for Plutonium.—Lord Rothschild, F.R.S.	789
Visualization of Mode Conversion of an Ultrasonic Beam in Fused Quartz.—V. J. Hammond and R. Carter	790
Scattering of Microwave Radiation by a Plasma Column.—Forrest I. Boley	790
Transformation of the α- and β-Forms of Calcium Sulphate Hemihydrate to Insoluble Anhydrite.—D. A. Powell	792
Estimation of Strontium in Biological Materials by means of a Flame Spectrophotometer.—Dr. G. E. Harrison	792
Observation of Transient Intermediates in Redox Processes by Variable Voltage Oscillo-polarography and Cyclic Voltammetry.—W. Kemula and Z. Kublik	793
Standard Potential at 25° C. of the Silver–Silver Chloride Electrode in Formamide.—Dr. M. Mandel and P. Decroly	794
Metabolism of Phenolic Compounds by the Broad Bean *Vicia faba*.—Dr. J. B. Pridham	795
A Paper Chromatography Method for the Determination of Suitable pH Values for the Extraction of Antibiotics.—Vladimír Betina	796
Exogenous Sources of Urinary Phenol and Indole Acids.—K. N. F. Shaw and J. Trevarthen	797
Rennin Coagulation of Enzymatically Dephosphorylated Casein.—Robert Y. H. Hsu, Prof. Laurens Anderson, R. L. Baldwin, C. A. Ernstrom and A. M. Swanson	798
'Solubilization' of a Cytochrome *b* Component from *Micrococcus lysodeikticus*.—F. L. Jackson and Valerie D. Lawton	799
Occurrence of Poly-β-Hydroxybutyric Acid in Aerobic Gram-Negative Bacteria.—Dr. W. G. C. Forsyth, Dr. A. C. Hayward and J. B. Roberts	800
Separation of Anthocyanin and Leuco-anthocyanin in Flowers of *Camellia japonica*.—Toru Endo	801
Incorporation of Orotic Acid-^{14}C and Lysine-^{14}C in Regenerating Single Nerve Cells.—S.-O. Brattgård, H. Hydén and J. Sjöstrand	801
Influence of Linoleic and Linolenic Acids on Symptoms of Vitamin E Deficiency in Chicks.—Prof. H. Dam, G. Kofoed Nielsen, I. Prange and E. Søndergaard	802
Inability of Epinephrine to protect Rhode Island Chicks against the Lethal Action of X-Rays.—Dr. M.-L. Beaumariage	803
Effect of Analogues of Glutathione on the Feeding Reaction of Hydra.—E. E. Cliffe and Dr. S. G. Waley	804
Adrenal Activity in Subjects with Complete Transverse Lesions of the Spinal Cord.—Dr. R. Robinson and Dr. A. F. Munro	805
Acetylcholine in Mammalian Neuromuscular Transmission.—K. Krnjević and R. Miledi	805
Metabolism of Coumarin and Related Compounds in Cultures of *Penicillium* Species.—David M. Bellis	806
Abnormally Low Ultimate pH in Pig Muscle.—R. A. Lawrie, D. P. Gatherum and H. P. Hale	807
Establishment of Permanent Cell Strains from Human Adult Peripheral Blood.—Dr. John Paul	808
Protoplasts of Group A Beta-hæmolytic Streptococci.—H. Gooder and W. R. Maxted	808
Calcium Exchanges of Ehrlich Ascites Tumour Cells of Mice *in vitro*.—Doreen Thomason and Raymond Schofield	809
Amœboid Motility of Fresh Ascites Tumour Cells.—Dr. Iwao Hirono	809
Cultivation of Isolated Embryonic Rat Tibiæ on a Chemically Defined Medium.—Miss Susan Heyner and Dr. J. D. Biggers	810
Pigments Present in Arms and Pinnules of the Crinoid, *Antedon bifida* (Pennant).—Miss E. J. Dimelow	812
The Finer Structure of the Galeal Lamella in Adult Mosquitoes.—G. Surtees	812
Dependence of Mitochondrial Swelling on Oxidizable Substrates.—J. B. Chappell and G. D. Greville	813
Effect of Low Temperature on the Vertical Distribution of Two Wood-boring Crustaceans.—Dr. N. Balakrishnan Nair and H. Leivestad	814
Trisaccharides of *Lolium* and *Festuca*.—Dr. Anna M. MacLeod and Dr. H. McCorquodale	815
Populations of *Agrostis* spp. Tolerant of Heavy Metals.—D. Jowett	816
Fleas of the Rat in Tanganyika.—J. G. Halcrow	817

"BELLING-LEE" MINIATURE *Unitors*

WITH 8 or 12 CONTACTS

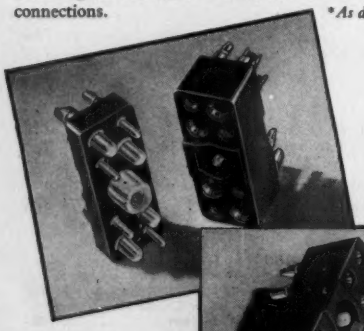
For the inter-connection of units in one assembly where space and weight considerations prevent the use of standard Unitors, we have designed and developed this new range of Miniature Unitors in the "Domino" series.

Moisture-and tracking-resistant nylon-filled phenolic material is used for body mouldings. Pins are of gold-plated* or silver-plated brass. Sockets of high grade beryllium copper, slotted to ensure good contact with pin.

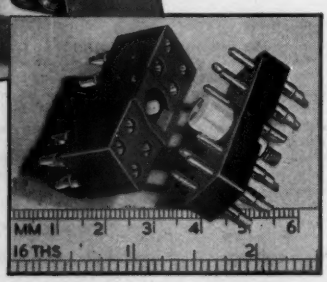
Each assembly has a shaped centre pin which polarizes the plug on engagement, and the back of this pin carries an 8BA stud for mounting the Unitor on chassis or panel.

Various pin combinations and arrangements are possible within the same chassis cut-out, to give a highly flexible method of obtaining an individual connection for each group of inter-connections.

**As distinct from gold flash.*



L.1387/P
L.1387/S



L.1388/P
L.1388/S

L.1387/P. PLUG, with four small and four large pins
L.1387/S. SOCKET FOR L.1387/P.
L.1388/P. PLUG, 8-pole (8 small pins)
L.1388/S. SOCKET FOR L.1388/P.
L.1389/P. PLUG, 12-pole (12 small pins)
L.1389/S. SOCKET FOR L.1389/P.

These miniature Unitors and other new developments are fully described in the Supplement (No. P490/H) to our General Catalogue: copies are available on request.

BELLING & LEE LTD
GREAT CAMBRIDGE ROAD, ENFIELD, MIDDX., ENGLAND
Telephone: Enfield 3322 · Telegrams: Radiobel, Enfield

GRIFFIN
Established 1820

JUST PUBLISHED

THE FUNDAMENTALS OF STATISTICAL REASONING

M. H. Quenouille, M.A. **169 pages. 24s. net**

Sets forth the underlying ideas and concepts which form the basis of statistics: it deals with "the principles rather than the proofs or the practices" of the science. Of interest to both the mathematician and the student of applied statistics. (*Statistical Monograph No. 3*)

THE ADVANCED THEORY OF STATISTICS (New 3-Vol.)

M. G. Kendall, Sc.D., & Alan Stuart, B.Sc.

VOL. I. DISTRIBUTION THEORY
456 pages. 84s. net

A new three-volume version of this important work is in hand. Vol. I above is now available, Vol. II—Statistical Inference and Statistical Relationship—will appear in 1960, and Vol. III—Statistical Planning and Analysis, and Time-Series—will follow Vol. II. Meantime the present Vol. II (3rd Edn. 1955, 72s. net) should be purchased.

Catalogue and prospectuses on application

CHARLES GRIFFIN & COMPANY LIMITED
42 DRURY LANE, W.C.2

LONDON

PRECISION INSTRUMENTS

Simple Type Table Spectrometer

This instrument is supplied at £35 complete with a 60° prism of dense flint glass, and is suitable for use also as a goniometer or refractometer by the 60° method.

If desired a camera, constant deviation prism and Gauss eyepiece can be supplied at extra cost.

Bellingham & Stanley Ltd.
DEPT. N, 71 HORNSEY RISE, LONDON, N.19
PHONE: ARCHWAY 2270

POLICY, PURPOSE AND PARTNERSHIP IN EDUCATION

SIR WILLIS JACKSON'S presidential address to Section L (Education) of the British Association on the partnership between industry and education laid emphasis on the conception of partnership in the sense that industry and education should each interest itself in the work of the other and, where appropriate, contribute to the work of the other, as well as fulfil the responsibilities which directly accrued to it within its own domain. Industry's responsibility was analysed in some detail from both points of view; but, while Sir Willis did not discuss to any extent the specific parts to be played by the schools, the technical colleges and the universities, for example, his address had a much wider interest and directed attention to aspects of technical and technological education which, as some other addresses at the British Association meeting have indicated, are recognized as of increasing importance. Like so many others, he insisted that the supply of well-qualified and well-trained teachers is the most crucial aspect of all our educational plans; but the partnership he advocates is put in its proper perspective, so that the responsibilities of the government and of the community itself can no less clearly be seen.

Sir Willis considers that industry has already gone some way towards convincing the schools that industry can offer those with a sense of vocation as satisfying a career as the 'learned' professions, which have traditionally attracted a dominant proportion of the ablest young people of Britain. This means, he believes, that industry is coming to be seen not merely in terms of machines and scientific and technical processes, but also in terms of men of character and stature, and of the opportunities it provides both for the exercise of scientific and technical skill and for co-operation in service and human relations. None the less, he points out that the importance of the supporting contribution has been insufficiently recognized, and that we have given too little attention to the educational needs of the much larger body of technicians and craftsmen. Here, too, technical change is not simply altering the balance of the labour force and increasing the numerical importance of the scientific, technological and technical staff, of the skilled men engaged on service and maintenance, relative to direct labour. It demands also increasing versatility, adaptability and capacity for understanding changing situations and points of view.

To reach the consequent higher level of scientific knowledge and attainment in an increasing proportion of industrial personnel and extended and broader education for all, industry, Sir Willis suggested, carries a considerable responsibility, and he refers with appreciation to the value of the work of the Industrial Fund for the Advancement of Scientific Education in Schools. Nor is this the only point at which his address reinforces Prof. F. A. Vick's argument about the contribution of the university to education in a technological age. He commented, for example, on the desirability, in day-time release from employment, for some time in attendance at technical colleges to be available for participation in corporate life within the college and for the continuance of general education. Reinforcing the recommendation of the Committee on the Supply and Training of Teachers for Technical Colleges over which he presided, he urged the need for more industrial concerns to take active steps to promote close contact with full-time members of the college staffs and to increase the absolute contribution made by part-time teachers; in this connexion he touches on the importance of status and of associating some such teachers both with advanced teaching and with the formulation of academic policy.

Here, as when he referred to the importance of bringing representatives of the highest ranks of industry into closer association with the governing bodies and advising committees of the technical colleges, Sir Willis indicated ways in which we can more effectively deploy the full strength of our existing teaching strength, rather than increase its numerical strength. It could well be said that concern with obtaining the fullest value both from the present educational effort and the existing scientific and technical manpower in Britain was one of the keynotes of this address. That, for example, is the implication of Sir Willis's reference to the importance of supporting technicians and craftsmen and no less of his suggestion that sixth-form grammar school boys should not be encouraged to remain at school to the age of eighteen with engineering as their objective, if they have shown little aptitude for the traditional subjects of grammar school curricula. Unless boys are capable of undertaking advanced work in physics and mathematics with fair promise of success at Advanced Level of the General Certificate of Education, Sir Willis believes it to be far preferable for them to enter industry at an earlier age and pursue their studies within the technical colleges.

This engineer's conception of fitness for the purpose, of choosing the tool for the job which pervades the whole address, is one that is all-important if the nation is to obtain full value from its present educational effort—already some £740 million a year and, according to Sir Alexander Fleck, needing to be increased to some £1,200 million—and it supplies the decisive reason for attention to the apprenticeship system, the discussion of which occupied a large part of Sir Willis's address.

It is here that he regards the responsibility of industry as most direct, and he was highly critical of the failure of the Carr Committee to go further than to recommend a purely advisory national apprenticeship council, the function of which would be to keep representative organizations of employers and workers informed of progress and to collect and disseminate information about those aspects of training common

to more than one industry. Something much more fundamental is urgently required, including considerable changes of attitude among both employers and trade unions, an extensive building programme and solution of a difficult staffing problem. The position is one that cannot be left to drift for, as once again we are reminded, the changing educational pattern means that from now on an increasing proportion of the boys of high natural ability who, in spite of deficiencies in their early training, have provided excellent craftsmen in the past, will remain at school beyond the age at which at present industry recruits its craft apprentices. A lowering in the quality of the entrants to craft apprenticeship is thus to be expected at a time when the raising, not lowering, of the standard of craft skill is imperative.

In this situation the deference of the Carr Committee to traditional practices and embedded prejudices on both sides could be disastrous, and such deference is the more disturbing in that Lady Gertrude Williams has already analysed the situation searchingly and constructively in her book "Recruitment to Skilled Trades". Sir Willis indicated the range of questions which her analysis has raised, but he declined to discuss them because he believes that individual opinion is unlikely to make any impact in the continued absence of a national body with executive powers and the financial means for exercising those powers effectively. Moreover, he pointed out that, so far back as 1944, the report of a conference of representatives of the Manchester and District Committees of the Engineering and Allied Employers National Federation, the Amalgamated Engineering Union, the major engineering institutions and the Regional Advisory Council for Technical and Other Forms of Further Education had recommended the establishment of a national engineering apprentice council as a central directing body, and of associated regional councils with defined responsibilities in their respective areas. These responsibilities for the organization and administration of training schemes included estimating the future needs of industry in the area and adjusting the intake of apprentices to training schemes accordingly; advising on the establishment, equipment, staffing and maintenance of apprentice training centres to meet the regional needs; co-operation with employers in placing apprentices on completing their courses; approval of facilities for further training; interest in the development of non-vocational interests; and the issue of certificates of competency on completion of apprenticeship. The report recognized the need for a detailed investigation as a basis for any such scheme, but equally the urgent need to face and solve the difficulties involved in implanting such proposals. It was confident then that it was within the power of Great Britain to ensure that its system of technical education, both in its teaching staff and in its buildings, was made adequate to the demands of the next decade.

A decade has been lost, but we need not be more pessimistic than in 1944 as to our ability to develop the necessary facilities if we are willing to apply the financial and other resources required, and resolute to deal firmly with whatever prejudices or obsolete practices obstruct such development. It means that the conditions laid down by Sir Willis Jackson must be faced frankly and resolutely handled as impartial inquiry and constructive thought may indicate. What duration of training, suitably organized and conducted, is really necessary for the different crafts, as against the universal five-year period from sixteen to twenty-one years of age for all crafts? Should the age of entry to craft apprenticeship be raised to permit the entry of boys who have pursued general education at school beyond the age of sixteen? Should early stages of training be carried out in training centres on a full-time basis where continued general education could also be provided, and if so whether such centres could be organized co-operatively by groups of firms on an adequate, nation-wide basis, or whether they must be provided by the local authorities? How to assess the numbers of recruits for the various crafts, and how to test competence—these are all questions demanding searching and impartial inquiry in the light of present conditions and trends, and resolute urgent action on the basis of such inquiry.

Finally, there is the question of finance. Whether a national fund should be provided jointly by the State, the local education authorities, employers and trades unions—and on what basis—demands inquiry, as does the question of how individual firms should be assisted to bear the cost of running their own training schemes. It can at least be predicted with confidence that whatever the basis to which such inquiry may point, it will offer a contribution to the economics of education out of all proportion to the direct expenditure involved. Running through the papers on varied aspects of education contributed to the Glasgow meeting of the British Association has been a note of concern that in the growing scale of expenditure on education, increasing care should be taken to see that the nation receives full value for that expenditure. Attention to this question of the training of technicians and apprentices will involve some further direct expenditure; but that expenditure is likely to be offset many times over by the elimination of the waste involved in the persistence of the present obsolete practices, quite apart from the direct gain in the more efficient and effective use of the more highly trained scientist and technologist whose work it is a primary function of the technicians and craftsmen to support and extend.

CHEMICALS AND CANCER

Mitotic Poisons and the Cancer Problem

By Dr. John J. Biesele. Pp. viii+214. (Amsterdam: Elsevier Publishing Company; London: Cleaver-Hume Press, Ltd., 1958.) 37*s.* 6*d.*

A FEW years ago, the editors of "Tabulæ Biologicæ" asked cytologists and geneticists in various countries to provide material for quantitative tables on the effects of mitotic poisons and chemical mutagens. Several, possibly all, of those approached

were of the opinion that the time for such a tabulation had not yet come in spite—or rather because—of a tremendous and bewildering wealth of data. While tabulation at this stage may do more harm than good by its false suggestion of orderliness and congruence in a field of much conflicting evidence, an impartial review was needed to summarize the present position and point out the most useful and promising lines for future experimentation.

Everyone interested in the subject of mitotic poisons—and this includes plant breeders and oncologists as well as cytologists, geneticists and biochemists—must be grateful to Dr. Biesele for having undertaken this task. The great number of literature references—nearly a thousand, including many German ones—shows what a tremendous mass of material has been compressed into less than 200 pages. It was not to be expected that this degree of condensation would result in an easily readable book, but there is no lack of clarity and precision. If, in spite of this, the facts are often confusing, this is not Dr. Biesele's fault. One only needs to look at the evidence for and against inhibition of deoxyribonucleic acid synthesis as the primary effect of alkylating agents, or at the description of the highly complex and variable interactions between purines to realize how far we are still removed from insight into the ways in which mitotic poisons act. It is natural that every group of investigators favours interpretations which agree with their own particular results, and thus conflicting theories have been built on conflicting facts. Dr. Biesele impartially reports both facts and theories and never falls into the error of facile over-simplification. A more personal point of view would almost certainly have made the book more readable, but it would probably have destroyed its prime value as an objective summary and source of references.

The only type of evidence which is almost wholly neglected is the purely genetical one. Like most cytologists, Dr. Biesele does not seem to recognize the fact that a genetically established chromosomal change is just as real as one seen under the microscope, probably more so. This bias has resulted in a somewhat incomplete report on the chromosomal effects of alkylating agents, and in the complete omission of formaldehyde as a substance which in *Drosophila* produces all known types of chromosome rearrangement. Incidentally, drosophilists would not agree that a chemical (3,6-disodium endohydrophthalate) which produced scarcely 1 per cent sex-linked lethals in *Drosophila* is "strongly mutagenic".

The data are grouped according to various types of mitotic effect: poisoning at prophase and interphase; poisoning of the chromosomes; poisoning at metaphase and later stages. Since these effects may overlap and many substances act in more than one way, a chemical classification might have been preferable; a good subject index, however, makes it easy to follow any particular substance or group of substances through the whole mitotic cycle. As indicated in the title, the importance of mitotic poisons in cancer therapy is stressed throughout the book. Dr. Biesele's own research on substances which act preferentially on tumour cells is here of special interest. The highly important role of mitotic poisons in cancer therapy, coupled with the fact that so far no really curative chemicals have been detected, adds great practical significance to this review.

C. AUERBACH

NAPHTHALENE

The Chemistry and Technology of Naphthalene Compounds

By Norman Donaldson. Pp. xv+512. (London: Edward Arnold (Publishers), Ltd., 1958.) 90*s*. net

THIS book is concerned with the preparation and manufacture of naphthalene derivatives, especially those, of course, involved in the manufacture of dyestuffs. Its most important feature can be illustrated by reference to the aminonaphtholdisulphonic acids: each of the thirty-six known isomers is listed together with at least an indication of its preparation, while the properties and manufacture of the more important, for example H-acid, are described in detail. The available information (industry broods over the more intimate) concerning such compounds seems to be assembled in this book, which, therefore, is valuable for reference purposes. Included also is discussion of the distribution of electrons in naphthalene and the mechanism of sulphonation, nitration, diazotization, diazo-coupling, the Bucherer reaction and other substitution processes. Unfortunately, these topics seem to place the author on unfamiliar ground, for there are comments of the type "However, negative substitution of amines also reduces their basicity, so that diazotisation velocity is decreased by the smaller proportion of amine salt available".

The book is not well written, the meaning of the text often being difficult to find. The difficulty is enhanced by the use of unexplained abbreviations, for example, E.s.d. in the table on p. 5 and the arrow which appears in the statement "Griess obtained the azo-dye aniline → naphthylamine in 1864" on p. 77, while its significance is explained on p. 85. The contents of the book have been assembled with insufficient attention to detail and there are more accidental mistakes than one expects to find in a book that costs 90*s*.

G. BADDELEY

COLLOIDS, OLD AND NEW

Organic Colloids

By Prof. Bruno Jirgensons. Pp. xiv+655. (Amsterdam: Elsevier Publishing Company; London: Cleaver-Hume Press, Ltd., 1958.) 85*s*.

IN this book of 655 pages the author attempts a complete survey of colloid science, with boundaries extending inside biological fields, with the object of producing a text suitable for non-specialists and readable by "students and research workers who have had basic courses in physics and organic chemistry". The emphasis is on "basic facts and relationships rather than on theory" and the treatment is essentially non-mathematical.

The author is at pains to fit modern macromolecular chemistry into the framework of classical colloid science, with the result that one occasionally encounters unusual combinations of old and new. There is a wealth of readable information in this book, necessarily without great detail, and the author has succeeded in saying something about almost every major topic in his field—in itself no small achievement.

Unfortunately, however, the text is marred by occasional inaccurate or misleading statements and by an apparently arbitrary selection on the part of the author of the material included. The following are

a few examples. On p. 37 we read "The life span of a free radical in the process of polymerization is estimated of the order of a few seconds only"—a misleading statement without further qualification. The polymerization of γ-methyl-L-glutamate N-carbonic anhydride in the presence of pyridine is not necessarily initiated by traces of water as implied on p. 45. The phenomenon described on pp. 316–17 and cited as an example of "replica polymerization" has, in fact, no connexion with the latter (see Waltcher, *J. Polymer Sci.*, **14**, 411; 1954). The section on synthetic polyamino acids (Chapter 25, section 4) is particularly inadequate. The chain-growth of polyamino-acids in the Leuchs reaction is said to be promoted by the presence of a polyamino-acid (the observation being attributed to Waley and Watson), with the suggestion that this is a further instance of replica polymerization. This is quite untrue; the preformed polymers used by Waley and Watson simply function as initiators (in the systems studied) by virtue of their terminal base groups, as stated clearly by these investigators; no mysterious "orientation effects" are involved. The α polypeptide of D-glutamic acid is not the capsular substance of *Bacillus anthracis* as might be inferred from p. 428. The important fact, mainly responsible for the recent rapid growth in interest in synthetic polypeptides, that these polymers can exist in crystalline α and β forms is not even mentioned. Finally, the uninitiated reader, looking at p. 474 and Figs. 125 and 126, will be left wondering whether silk fibroin is, after all, a β protein. Defects of this kind detract considerably from the value of the book as an introduction to macromolecular chemistry.

The first 16 chapters (comprising Part 1, 280 pages) deal with fundamental principles and general methods of preparation and study, while the remaining 18 chapters (Part 2) deal with selected groups of organic colloids. The scope of this part is illustrated by the following selection of chapter headings: Macromolecular hydrocarbons: Cross-linked space polymers; Cellulose and similar polysaccharides; Globular proteins; Nucleic acids, nucleo proteins and viruses; Colloid phenomena in cells and tissues; Colloid-chemical characterization of biocolloids in disease; Micellar detergents, dyes and pigments.

The book is well produced and misprints are few.

C. H. Bamford

VIRUS AND VEGETABLE

Investigation of Virus Diseases of Brassica Crops

By Dr. L. Broadbent. (Agricultural Research Council Report Series, No. 14.) Pp. vii+94+8 plates. (Cambridge: At the University Press, 1957.) 15*s.* net.

BRASSICA crops are susceptible to two virus diseases, cabbage black ringspot and cauliflower mosaic, which, periodically, have caused great loss to the industry. The years 1948–50 saw more than usually severe outbreaks in crops of cauliflower and broccoli. In 1950, Dr. Broadbent, with the collaboration of many members of the National Agricultural Advisory Service and others, began to expand work already in progress at Rothamsted Experimental Station with the view of learning more about these diseases, especially those aspects relating to their control. Work continued for the next five years, and in "Investigation of Virus Diseases of Brassica Crops" it is reported in detail.

The contents of the book may be divided into two main parts, one dealing with response to infection and the other with spread of virus. The first of these includes a study of the response of a wide range of hosts including many different varieties of cauliflower, cabbage, and other brassicas, and the effect of factors such as strain of virus, weather conditions and manuring on symptoms. It also includes a useful list of plants susceptible to each virus. The other part of the book deals first with the process of transmission of the viruses, including the relative importance of different species of aphids in the natural spread of the diseases and, secondly, with their spread into and within the seedbed and crop. Appended is a short account of the two viruses transmitted by flea-beetle causing turnip yellow mosaic and turnip crinkle.

The recommendations for control which emerge from the study are similar to those advocated in the past. However, the experimental scrutiny to which they have been put should enable them to be applied with greater confidence and effectiveness. It is heartening to learn that commercial varieties of cauliflower differed both in the readiness with which they became infected and in the severity of the symptoms displayed after infection. This encourages the hope that varieties with increased resistance or tolerance may be bred for use in the future.

The inclusion of a large amount of experimental detail tends to slow the reader, but this is largely off-set by good lay-out. The production and most of the plates are excellent. Combining as it does original observation and reference to the published work of others, it will be of great service to those requiring an up-to-date and full account of the virus diseases of brassicas.

J. H. Hitchborn

AN IMPORTANT HERBARIUM

The Sloane Herbarium

An Annotated List of the *Horti Sicci* composing it; with Biographical Accounts of the Principal Contributors, based on Records compiled by the late James Britten. Revised and Edited by J. E. Dandy. Pp. 246+2 plates+96 facsimiles of handwritings. (London: British Museum (Natural History), 1958.) 147*s.*

AMONG the historical collections at the British Museum (Natural History), the Sloane Herbarium is one of the most important. A few years ago the director, Dr. G. R. (now Sir Gavin) de Beer, published a biography of Sir Hans Sloane, under the title "Sir Hans Sloane and the British Museum" (published for the Trustees of the British Museum by the Oxford University Press, 1953). Sloane is rightly regarded as the founder of the British Museum and his herbarium is probably the most extensive accumulation of botanical specimens of the seventeenth and early eighteenth centuries extant or ever made. The dried specimens forming the *Horti Sicci* are in bound volumes (*H.S.* 1 to 334), many one to a volume, but some bound together in twos or threes, five not present, one (*H.S.* 334) in two volumes, and eight with extra numbers giving a total of 265 volumes.

The late James Britten, who for thirty-eight years was an assistant in the Department of Botany at South Kensington, prepared an account of the Sloane Herbarium, which was unfinished at the time of his death and was not published. This account

has now been revised and edited by Mr. J. Dandy, the present keeper of botany, and, with an introduction by Mr. Spencer Savage, formerly librarian and assistant secretary to the Linnean Society of London, is printed by order of the Trustees of the British Museum.

In a preface, Mr. Dandy modestly refers to the amount of revision he had to undertake on Britten's manuscript. This involved a page-by-page examination of all the volumes, many additions to and modifications of the original text, and modernization of botanical and other names. He emphasizes that the book is not a catalogue of the specimens in the Sloane Herbarium but a guide to its contents with historical information about the collectors. To taxonomists the type-specimens present in the Herbarium are of greatest importance and a complete list of these has not yet been prepared.

Mr. Savage revised and arranged the manuscripts left by James Britten, selected the facsimiles of handwritings, of which 96 are reproduced, and prepared an introduction. In this he gives some interesting generalizations regarding the collections that make up the Sloane Herbarium. Specimens were obtained from as many sources as possible with the aim of accumulating more new and rare ones than anyone else. No serious attempt was made to determine the plants taxonomically and there was so little arrangement that Linnaeus, who saw the Sloane Herbarium on his visit to England in 1736, wrote that it was 'in complete disorder'. Later there were attempts to refer the specimens to Ray's "Historia Plantarum" by Sloane himself and by Dr. Johann Amman, who became his curator. Since the Sloane Herbarium is made up of specimens from many collectors and previous owners the correct attribution of handwritings is of great importance, and Savage did good service in identifying many of these. Further notes regarding the Sloane collection are contained in the few pages of general talk by Britten.

The main bulk of the volume consists of two parts: an annotated list of the *Horti Sicci* composing the Sloane Herbarium and biographical accounts of contributors, together with some information about their specimens. Among the more important collections in the *Horti Sicci* are those of Sloane himself made in Jamaica and on the voyage thither (1687–89); those of Bartram, Catesby, Courten, Cuninghame, Kaempfer, Kamel, Kiggelaer, Petiver, Plukenet, and Vernon. There are many collections of British plants, including some first records for the British flora, and plants from gardens of which those of Philip Miller from the garden of the Apothecaries' Company at Chelsea are probably outstanding in taxonomic importance, and those from the garden of the Duchess of Beaufort, at Badminton, Gloucestershire, and from Boerhaave, Leyden, are of considerable horticultural interest. While the plant specimens are not listed the countries of origin of many of the collections are given.

The second part is an alphabetical list of those persons whose collections (first- or second-hand) make up the Sloane Herbarium together with longer or shorter accounts of their lives and of their contributions to the Herbarium. There is much useful and interesting information for the botanical historian here and numerous bibliographical references are given. The usefulness of the book is increased by an index to geographical localities and another to botanical names. The facsimiles of handwritings, mentioned above, are well reproduced as plates at the end of the volume.

W. B. Turrill

THE *SIBOGA* POLYZOA

The Polyzoa of the *Siboga* Expedition

By the late Sir Sidney F. Harmer. Part 4: Cheilostomata Ascophora, II (Ascophora, except Reteporidæ, with additions to Part 2, Anasca). (Monographie, 28*d*, Uitkomsten op Zoologisch, Botanisch, Oceanographisch en Geologisch Gebied verzameld in Nederlandsch Oost-Indië 1899–1900 aan boord H.M. *Siboga*. Uitgegeven door Dr. Max Weber en Prof. Dr. L. F. de Beaufort.) Pp. xv+641–1147+plates 42–74. 240 guilders.

WHEN Sir Sidney Harmer died in 1950 he had published since 1885 a long series of papers on the Polyzoa, including three reports (1915, 1926 and 1934) on the Polyzoa collected on the *Siboga* Expedition. The present volume, by far the largest of the reports, completes his studies on those extensive collections. Apart from three and a half pages, it deals with the Ascophora. Harmer had intended to divide these into two divisions, the Ascophora Imperfecta and the Ascophora Vera, based on the nature of the compensation-sac. The distinction is an important one, but, although his typescript explicitly referred a few families to the former, it was not clear (for reasons given below) whether all the remaining genera were to be included in the Ascophora Vera or only the majority of them. These 'remaining genera' are divided among thirty families, with eleven others unplaced. Altogether, species belonging to 84 Ascophoran genera are described, and numerous other genera discussed. The systematics and synonymies of the genera and species are dealt with fully.

As is usual with Harmer's work, the Polyzoa were not studied only from that point of view, however. Careful and detailed analyses of the morphology of the zoaria of a number of species are also given—an outstanding example is that of *Siphonicytara formosa* Harmer, and those of the species of *Conescharellina* are also noteworthy.

Previous to the *Siboga* Expedition, very little was known of the Polyzoa of the East Indies, and nothing of their deep-water representatives there. Now, Harmer's work has revealed a rich and varied Polyzoan fauna, collected from the shore-line down to abyssal depths, though the majority come from less than 90 metres. Some of the species have wide bathymetric ranges, for example, *Celleporaria tridenticulata* (Busk) was found at depths from 0–397 metres, and *Agalmatozoum decussis* (Canu and Bassler), previously recorded from 249 fathoms, was found at depths between 535 and about 3,000 metres.

As was to be expected, the East Indian Polyzoa show many similarities to those of the Philippines, although they include numerous species not described from the latter area by Canu and Bassler in 1929. In his comparisons, Harmer not only made full use of a collection of Philippine Polyzoa which those authors had sent to the British Museum (Natural History), but also of others, such as the Haddon Collection from Torres Straits and, more particularly, the Thornely Indian Ocean Collections. As a consequence, we now have full and easily usable synonymies and descriptions of the Polyzoa of the Red Sea and Indian Ocean. These are amplified by a wealth of clear and beautiful figures, such as characterize all Harmer's works, figures all drawn by the author himself. If any criticism of the plates can be

made, it is that the figures on some of them are somewhat overcrowded, and the lettering is at times too faint.

An interesting outcome of the investigations is the light thrown on the provenance of the unlocalized specimens, named and figured by Audouin and Savigny, which were collected during Napoleon's invasion of Egypt. The close agreement between them and the Indonesian material indicates that they came most probably from the Red Sea coast rather than the Mediterranean.

There are a few nomenclatorial surprises in the work, such as the demonstration of the priority of *Margaretta* Gray over the well-known *Tubucellaria* d'Orbigny, and of *Dakaria* Jullien over *Watersipora* Neviani. The replacement of *Catenicella* auctt. by *Vittaticella* Maplestone may also be regretted but is correct. In three other instances, however, Harmer, always hitherto a stickler for the sanctity of the International Rules of Zoological Nomenclature, strays from the path of virtue—his substitution of the type species of *Celleporaria* Lamouroux and *Schismopora* MacGillivray by others cannot be accepted, and his statements on the taxonomy of *Hippoporella* Canu are open to serious question.

These are small blemishes, however, in a volume which completes a remarkable life-time's work on the Polyzoa. Harmer, of whom there is a striking portrait in this last volume, carried out his extensive studies with a scrupulous regard for scientific accuracy; he expressed his observations and conclusions with a clarity and fullness that make them easy to comprehend and use. In the result, his *Siboga* Reports rank as the finest contribution to our knowledge of the Polyzoa since G. Busk's classic *Challenger* Report in 1884.

Finally, we must refer to the parts played by Dr. Anna B. Hastings and Dr. L. F. de Beaufort (the editor of the reports) in the present publication. Harmer had mostly completed his work when the Second World War broke out, and the printing of the plates had already started in Holland in 1940. By the end of the War, Harmer's failing health had stopped his further work, and at his request Dr. Hastings was entrusted with seeing the report through the press. It must have been no easy task, as the typescript was not as ready for publication as had been supposed. Nevertheless, with her wide knowledge of the group, she was able to co-ordinate and frequently check the text. At the same time, she studiously avoided modifying Sir Sidney's observations and opinions: although she has made several pertinent comments, they are all clearly indicated. For all this, the zoological world owes a great debt to Dr. Hastings, as well as to Dr. de Beaufort, who wisely decided after the War to carry on with this important publication.

GEOLOGICAL NOTIONS OF BERNARD PALISSY

The Admirable Discourses of Bernard Palissy. Translated by Aurèle La Rocque. Pp. x+264. (Urbana, Ill.: University of Illinois Press, 1957.) 5.50 dollars.

THE phenomena of the Earth's crust attracted attention long before the foundations of geology were laid at the beginning of the nineteenth century, but though pregnant with possibilities, the early observations had little effect upon contemporary thought and never came together as parts of a developing theme. Even to-day they are usually cited as novel notions anticipating present-day knowledge.

For this reason, an English translation of the "Admirable Discourses" of Bernard Palissy, a book that is neither readily accessible, nor easy to read in its sixteenth-century French, is very welcome because his work in ceramics and enamel is better known than his contributions to geological thought. It shows us the working of the mind of one who, had he lived two centuries later, would have contributed towards the emergence of geology as a science, and although the limitations of his knowledge sometimes led him to conclusions that we now know to be wrong, in those in which he was right he showed perspicacity beyond that of his contemporaries.

In a short introduction, the translator summarizes the little that is known about Palissy's early life, reviews his later activities against the political and religious background of his time, and appraises his contributions in the field to which the book relates.

Palissy spent much of his time at Saintes, north of Bordeaux, and although by no means uneducated, had little formal training; what he had did not include Latin and Greek so that his opinions were not greatly influenced by classical and medieval writers. For some years after 1575 he gave lectures on natural history in Paris, but his unorthodox scientific views, added to his adoption of the reformed religion, led to periods of persecution and his ultimate death in the Bastille.

The breadth of the field he made his own is indicated by the title of the book which embodied the material of his lectures—"Discours Admirables, de la nature des eaux et fonteines, tant naturelles qu'artificielle, des metaux, des sels et salines, des pierres, des terres, du feu, etc.". It is written in the form of a dialogue between 'Practice' and 'Theory'. Practice (himself) instructs Theory, who puts the questions or challenges the answers. When Theory called him a great dolt for doubting the philosophers who argued that the water of springs was provided by the sea, he replied that if that were the case spring water would be salty (which it is not) and the sea would have to rise as high as the highest springs (which it does not). "Don't you know," he continued, "that the land is full of holes, cracks and abysses through which the water coming from the sea would come out on the plains before it could climb the mountains? Now fetch your Latin philosophers to give me a contrary argument that would be as easy to grasp as the one I put forward."

He regarded springs as being supplied by rain water that had percolated down through sandy material until it reached an impervious bottom that could hold it up, and he was the first to give a correct explanation of artesian wells. He denied that fossils were relics of the Biblical flood, but thought them to be the remains of animals that had lived where the fossils are found, from which it followed that there had once been water where there was now dry land. His knowledge was not extensive enough to enable him to follow the argument to its logical conclusion and to suggest that land and sea had changed places, and he explained the phenomenon by postulating lakes that had drained away.

He recognized that rocks were not formed all at the same time, described an auger with a hollow tube at

the tip and handles of different lengths for bringing up samples of soil from various depths, and observed that crystals assumed characteristic angular forms, just as it was "given to plants to keep a certain order, as you see that roses and gooseberries form prickly spines for their defence".

The interest of the "Discourses" lies not only in their revelation of what Palissy noted and inferred, but as much in what he failed to realize by reason of the limitations of his own observations and his lack of knowledge of what others had discovered in the same field.

F. J. NORTH

QUANTUM MECHANICS

The Principles of Quantum Mechanics

By Prof. P. A. M. Dirac. Fourth edition. (International Series of Monographs on Physics.) Pp. xii+312. (Oxford: Clarendon Press; London: Oxford University Press, 1958.) 35*s*. net.

THIS is the fourth edition of a work too well known to require reviewing in detail. Dirac's "Quantum Mechanics" must surely still have its place on the shelves and in the heart of every serious student of quantum theory. For as long as the reviewer can remember it has been part of the scheme of things—quantum theory absolute as first conceived by the author and as no one but he could set it forth. What other subject possesses a text-book which combines in this way such definitive character and yet such dissimilarity in form from all other treatises on the subject? Here is Dirac's "Quantum Mechanics" and there Pauli's "Handbuchartikel", scarcely recognizable as dealing with the same subject and, as it were, illustrating the principle of complementarity; the serious student could not do better than study them side by side, as was the reviewer's good fortune to do in his student days.

For the second and third editions the author rewrote much of the text, but in doing so, did not fundamentally change the spirit of the work, even when in the third edition he introduced the entirely new *bra* and *ket* notation. In this present edition the main body of the book has undergone almost no change, but the concluding chapters on relativistic quantum theory have been rewritten and altered very profoundly. Curiously enough the theory of the Dirac electron as expounded by Dirac and the developments following it have always seemed to the reviewer to be the least satisfactory parts of the earlier editions of this book. Just because the relativistic theory itself has not arrived at the same state of conclusiveness as the rest of quantum theory, the characteristic Dirac style is inevitably less satisfying when dealing with it. It has also been apparent for some time now that the treatment of the interaction of the electron with the electromagnetic field given in the third edition, far from being definitive, showed Dirac to be turning away from the main stream of current thought on the subject.

This has now been recognized by the author, who admits that "a quantum electrodynamics which demands conservation of the number of charged particles is . . . out of touch with physical reality". The extensive modifications made in consequence are most welcome (as is also the minor change to the more usual sign convention for the Hamiltonian and the α-matrices). The student of the new edition, instead of being ultimately deflected from the main stream of modern research, is now guided to a point where this stream is just within sight. However, he is not cast adrift in its turbulent waters. True to his principles to the last, the author stops short of all considerations which cannot be developed with full logical consistency. Renormalization, for example, is not even named and only the last half page makes reference to what occupies so many quantum physicists to-day: "People have succeeded in setting up certain workable rules", says the author, but in spite of the success of these rules in interpreting experiments, he refrains from discussing them, pointing out (with full justification) that they "do not fit in with the logical foundations of quantum mechanics", and leaving it at that.

At first reading one may regret the omission of so much important matter, but on reflexion would one not agree that this is as it should be? Dirac stands for perfection and where perfection is not yet possible, let others take over.

N. KEMMER

COMPRESSIBLE FLOW IN HIGH-SPEED AERODYNAMICS

High-Speed Aerodynamics (Compressible Flow)

By Prof. Elie Carafoli. Pp. 702. (London and New York: Pergamon Press, 1957.) 100s. net.

THE book is a translation of a Roumanian publication entitled "Aerodinamica Vitezelor Mari", edited by Editura Academiei R.P.R., Bucharest, and follows an earlier book on incompressible flow published by the same author. The latter has been translated into German and Russian but not, so far as I know, into English. In this book on compressible flow, Prof. Carafoli attempts to give a comprehensive account of subsonic and supersonic aerodynamics and in fact states in the preface, "we have endeavoured to deal with some present-day problems relating to compressible fluids both in their theoretical aspect and more particularly in their application to current problems of aviation".

While the author has gone a long way towards achieving this objective, he has not, in my opinion, been entirely successful. The book is mainly mathematical and contains much useful theoretical information on one- and two-dimensional flows of perfect fluids, but other aspects are given inadequate treatment, particularly those for which theory is not fully developed. In consequence the book lacks balance, and pays more attention to solved problems than to a discussion of those which are difficult to treat theoretically. For example, it contains little of practical value on methods of estimating wing loading in subsonic flow, whereas the corresponding problem in supersonic flow is dealt with at much greater length. Even so, attention in the latter case is mainly given to delta wings, for which mathematical solutions exist. No mention is made of Evvard's method, and the problem of estimating loading on wings of general plan form is not covered as adequately as it might have been. Hypersonic flow is not considered, and the book concludes with a section on unsteady flow theory which is no more than a brief and elementary introduction to the subject.

Progress in aerodynamic research is so rapid nowadays that a book which is up to date when written is inevitably much out of date, through no fault of

the author, by the time it is printed and issued. Most of the few references in the present book refer to work that was done before 1954. Although the book is lacking in some respects, particularly in the practical application of theory, it could be read with profit by any student of aerodynamics who is mainly interested in the mathematical solution of compressible flow problems. From the general reader's point of view, however, it could be greatly improved by the inclusion of more experimental data for comparison with theory, and fuller discussion of problems that are not amenable to theoretical treatment. An author's and a subject index would also make it more useful for reference purposes. W. P. Jones

NUCLEAR THEORY

Shell Theory of the Nucleus

By Prof. Eugene Feenberg. Second printing. (Investigations in Physics, 3.) Pp. xi+211. (Princeton, N. J.: Princeton University Press; London: Oxford University Press, 1955.) 4 dollars.

THIS volume is the third production of the Princeton University Series "Investigations in Physics". When it was first published in 1955, the covers were of paper and the style of print was that of typewritten stencils, both features suggesting that serious effort had been devoted to keeping the price to a minimum. The actual cost was rather less than thirty shillings, which was impressively low for a specialized American text.

A second printing has now been made without any change in textual content, style or price. (Even typewriting errors are reproduced.) Unfortunately, between the two printings, the book has suffered the usual fate of expositions on topics in the front-line of research, and has become dated. Even when the book first appeared, it could not be said to provide an adequate discussion of all important branches of the subject-matter. This is much more true now as a result of the publication of many new papers on nuclear shell theory in the past three years. The book is still a good guide to certain classical aspects of shell theory, but there are a roughly equal number of topics which are scarcely mentioned. In particular, there is little mention of the modification of the theory that has been made necessary by the discovery that many nuclei are not spherical but egg-shaped (the strong-coupling version of the unified model of Bohr and Mottelson). No space is given to the work of Brueckner and others on the problem of why the shell theory failed to explain nuclear binding energies. Most surprising of all, only very brief reference is made to the work of Inglis and Elliott on the intermediate coupling version of the theory, which results in some of the most convincing and detailed agreement with experiment. Preliminary reports on these items appeared in 1953 and a great amount of work has since been published. It is regrettable that the production of the Princeton Series, although admirable in many ways, apparently lacks the flexibility necessary to enable a dated text to be revised.

The book opens with a survey of the evidence for shell structure in the nucleus, then presents the single particle model, and follows this by successive chapters on the comparison of the model with electromagnetic moments, electromagnetic transitions (isomeric states), and beta transitions. The latter chapters contain tables of data which are not complete in view of recent results but give a fair picture of the situation. The last chapters give discussions of particular shell-theory configurations and of collective vibrations of nuclei. In several places, nuclei lighter than neon are discussed in terms of j–j coupling, although it is well established that this is a very poor approach to such nuclei.

A. M. Lane

TRANSISTORS

An Introduction to Transistor Circuits

By E. H. Cooke-Yarborough. Pp. xii+154. (Edinburgh and London: Oliver and Boyd, Ltd.; New York: Interscience Publishers, Inc., 1957. Published in collaboration with the United Kingdom Atomic Energy Authority.) 15*s*. net.

An Introduction to Junction Transistor Theory

By Dr. R. D. Middlebrook. Pp. xxiv+296. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1957.) 68*s*. net.

Transistor Circuit Engineering

Edited by Richard F. Shea. Pp. xx+468. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1957.) 96*s*. net.

UNTIL recently, little of the vast amount of information published about transistors had come by way of books, with the result that many newcomers to the transistor field have found the going somewhat heavy. Now, it appears that the drought is being followed by a deluge and it is interesting to see how three of the recent books help to produce a more balanced literature.

All three books are primarily concerned with the application of transistors, although Middlebrook's should also appeal to the designers of transistors. Cooke-Yarborough and Middlebrook intend their books as introductions, each author choosing an approach which is based on his own interests and experience. Shea and his co-authors, on the other hand, set out to give detailed guidance to the design of a wide range of transistor circuits.

After describing, with commendable brevity, how it works, Cooke-Yarborough considers the transistor as a circuit element. He discusses its use in various circuits, in each case carefully explaining what the transistor does, an approach which will help the reader when later he has to solve his own transistor problems. In view of Cooke-Yarborough's earlier contributions, it is not surprising that non-linear circuits take pride of place. Despite this limitation, the book should still appeal to both students and practising engineers who are looking for a readable introduction to transistor circuits. Some may find the unusual symbolism irksome, but the clarity of Cooke-Yarborough's writing should more than enable them to find the capacity for forgiveness.

Middlebrook's main interest centres around the transistor as a linear amplifier. However, the first section of the book deals with transistor physics, both qualitatively and quantitatively. This is followed by a section on electrical characteristics, leading to the derivation of equivalent networks for transistors. Much of the third, and final, section is taken up with a particular equivalent network. Engineers who specialize in the design of transistor linear amplifiers will find in this book a thorough theoretical background to the transistors themselves. Many others, including the designers of transistors, will find this a

useful book, in which the subject-matter has been well planned and ably presented.

Shea's book covers most kinds of transistor circuits, and is in many respects an enlarged and more advanced version of another book which he edited a few years ago. Written by a team of specialists, it naturally contains a wealth of practical information. However, it is not always up to date, a weakness that is inherent in a reference book that covers a rapidly developing subject. Nevertheless, it will be welcomed by many practising engineers, particularly by those whose work covers the design of a wide range of circuits. In addition to detailed studies of circuits, it contains a chapter describing how some of these can be fitted together to produce a practical equipment.

Without doubt each book fulfils a need and helps to bring a fascinating subject within closer reach of electrical engineers.

D. D. JONES

DETACHMENT RELINQUISHED

Personal Knowledge

Towards a Post-Critical Philosophy. By Prof. Michael Polanyi. Pp. xiv+428. (London: Routledge and Kegan Paul, Ltd., 1958.) 42*s*. net.

A CHARACTERISTIC feature of the contemporary approach to science is that, both as a discipline and as a substantial part of knowledge, it is objective, and basically impersonal. These features are commonly considered to be virtues. For some time, and in several quarters, doubts have been cast upon this view: now in this monumental work Prof. M. Polanyi adopts a position completely rejecting the whole ideal of scientific detachment. There must be such a thing as the conception of knowing, and it is just this which he sets out to modify. Indeed, his title, "Personal Knowledge", demands some reconciliation, otherwise the two words appear contradictory. For his machinery, the author turns to *Gestalt* psychology. It is true, as he says, that scientists have shied at the philosophical implications of the theory: for himself he embraces them, prepared for the consequences. Naturally, these are likely to be more far-reaching in the biological sciences than in the domain of physics and mathematics. Otherwise expressed, the urge to detachment has done more harm in the former sphere: in the latter, at any event in recent years, formal impersonality has become somewhat disregarded. The late E. S. Russell (whose name, by the way, does not figure in the excellent index) was something of a pioneer in his "Directiveness of Organic Activities", and reached much the same conclusion, but by rather different paths.

This use of the *Gestalt* concept is vital, for, as is common knowledge, its outstanding principle is that of integration. Otherwise, Prof. Polanyi would have found himself inhibited from achieving what is perhaps the summit of his endeavour, namely, to link all purposive awareness to value. Axiological frustration may well be one of the deepest ills of our epoch; here at least is a system of methodology capable of resolving the paradox of increasing power with diminishing faith. The fact is that nobody but the author could possibly have written this book, or anything like it. The passionate element in the very act of knowing is not something subordinate; it is of the essence of creative ability. With it goes the coupling—more and more demanded as our mental process deepens—between the sciences and the humanities, if man is not to flounder in a bog, largely of his own making. Meanwhile, it is perhaps as well to face the essential difference between science and technology. Both need originality, which is therefore not a discriminant. What the scientist does is to probe more deeply into the nature of things than other people: the technologist, however, is quick to see how phenomena can be turned to practical ends, which involves the assessment of a host of factors entirely irrelevant to the scholar as such.

Under the general heading of "Commitment", several matters are discussed which reveal the quality of the author's mind, and the grasp which he has obtained over his subject. In particular, one may note the high state of stress which accompanies the solution of a major problem. It is only possible to experience this if one believes that the question has an answer: in the event of success, there is rejoicing in discovery and more rejoicing in accepting it as true. This state of affairs is akin to the late Prof. E. A. Milne's "logical pressure", which he described as a kind of pre-condition for great discovery. (The immediate condition is slightly different, and contains certain historical and personal elements which together may produce simultaneous effects, or a near-miss, as the case may be.)

Over and above all this comes the struggle to gain agreement that something fundamentally new is both valid and intellectually coercive. An example would be the non-Euclidian geometries of Bolyai, Lobatschevski and Riemann. Again (pp. 308–9), we are reminded how great are the responsibilities of commitment when a judge decides a novel case within the framework of the existing law. Perhaps it is not wholly an accident that several high wranglers have achieved distinction on the Bench: men who have been through the rigours of mathematical manipulation, but who have not lost sight of the artistry of logical processes.

The relation between the axioms discovered by Gödel and logical inference machines has a direct bearing upon the current problems of automation in general. The crux of the matter would appear to be the extent to which the rules of any art can be specified. But any skill or connoisseurship, which is unspecifiable, cannot be fed into a machine. It looks as if it is just this class of human properties which forms the entire basis of Prof. Polanyi's thesis, namely those generated by the mind in its passionate search for truth. It is in this sense that "the powers of the mind exceed those of a logical inference machine".

Towards the end, the author introduces his concept of a heuristic field, with its analogues of lines of force, which "should stand for *an access to an opportunity. . . .*" It is significant that the greatest artists of the Italian rennaissance recognized this almost intuitively, when (as X-radiography has revealed) they made tentative outlines, perhaps with a blunt instrument, to the limbs of their figures before committing themselves to the final form. In the same way, Prof. Polanyi has spent some nine years in his majestic 'groping' for the ultimate verities, and thus shares in the wisdom of the ages "as one that traceth".

F. I. G. RAWLINS

Veterinary Protozoology

By U. F. Richardson and Dr. S. B. Kendall. Second edition, revised. Pp. xii+260. (Edinburgh and London: Oliver and Boyd, Ltd., 1957.) 22*s*. 6*d*.

ALTHOUGH protozoal infections of domestic animals are of the greatest economic importance, especially in tropical and warm countries, there are scarcely any reliable English text-books of protozoology covering this field to which students and practitioners of veterinary medicine can turn for information. As the first edition of the book under review, written by the senior author some ten years ago, suffered from a number of defects, it was hoped that the second edition, now produced by the joint efforts of two authors, would be considerably improved. The results are, however, somewhat disappointing, and the presentation of the subject-matter is uneven.

The general morphology and cytology of the protozoa, as well as their physiology, host-parasite relations and classification, are dealt with rather briefly in an introductory chapter, which is followed by six chapters on the flagellates, among which the trypanosomes rightly occupy a prominent place. Here it may be noted that the definition of certain inner structures (blepharoplast, basal granule, parabasal body and kinetoplast)—here, and in the introduction—are contradictory and confusing. A short chapter on *Histomonas* and entamoebae is followed by four chapters on the Sporozoa (especially coccidia and piroplasms). These are adequately dealt with, but more space could have been given to toxoplasmosis. A special chapter is allocated to ciliates (*Balantidium*) and an assembly of protists of uncertain systematic status, followed by chapters on chemotherapy and techniques.

In all sections, the description of the pathogenic parasites and their life-history is accompanied by an account of the diseases caused by them, and their treatment. These form the most valuable part of the book, whereas the description of some of the species of protozoa is not sufficiently accurate or clear for their differentiation. Furthermore, the parasitological diagnosis of some infections would have been facilitated by the provision of more and better figures, and by reference to the existing ones in the text.

C. A. Hoare

Nuclear Radiation in Food and Agriculture

Edited by Prof. W. Ralph Singleton. (The Geneva Series on the Peaceful Uses of Atomic Energy.) Pp. xii+379. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1958.) 64*s*.

THIS book is one volume of a series being published to cover various parts of the Geneva Conference on the Peaceful Uses of Atomic Energy. The volume under review is devoted mainly to those portions of the Conference which are concerned with food and agriculture. Each chapter of the book is a paper from the Conference and has therefore already been published by Columbia Press in the Proceedings of the Conference.

The editor—Prof. Singleton—has selected 28 papers for this book, from nearly 100 which were on appropriate subjects. The ones he has chosen are either reviews, such as "Use of radioisotopes in soil and fertiliser studies" by A. L. Dean, or specialist papers on important topics such as "Genetic erradication of the Screw-worm fly" by Bushland *et al.* In this way the editor has produced a book which should appeal to a wide range of readers.

The majority of the papers in the book refer to work with plants rather than animals. This editorial choice was made because the papers involving animals were mainly physiological in nature, rather than of general agricultural interest. There is, however, a section of six chapters on genetical and biological hazards of radiation, some of which are mainly relevant to humans. These latter papers could have been omitted since they are not primarily of agricultural interest and can be found elsewhere. It might then have been possible to have included other more relevant papers. Nevertheless, the book covers much important work. There are eight main sections dealing with nuclear radiation in fields as diverse as the use of radioisotopes; photosynthesis; plant physiology, pathology and cytology; soils and fertilizers; crop improvement; food sterilization; in addition to the subjects already mentioned.

A further feature is a list of relevant papers including those which were not selected for publication in the book. This adds greatly to its value.

Lorna J. Lilly

National Libraries of the World

Their History, Administration and Public Services. By Dr. Arundell Esdaile. Second edition, completely revised by F. J. Hill. Pp. xv+413+12 plates. (London: Library Association, 1957.) 44*s*.

THE second edition of Dr. Esdaile's book describes some thirty-two of the national libraries of the world, the growth of their stocks and the development of their services and the effect of the Second World War. Admirably presented and produced, it is representative rather than comprehensive, and the selection has been determined by such considerations as fame, historical interest or administrative significance, and also with a view to geographical distribution. The British Commonwealth is represented by the British Museum and the National Library of Canada, with short notes on the National Library of Scotland and the National Library of Wales, the National Library of the Commonwealth of Australia, Canberra, the South African Public Library, Cape Town, and the State Library, Pretoria, and the United States of America by the library of Congress. That list also indicates the key importance of the notes on the place of each library in its national system, though even here a note on the British Museum which does no more than refer to the National Central Library can scarcely be regarded as adequate. The value of the book to a general reader and to many librarians would be enormously enhanced if these notes could be expanded to include a brief account of the several national systems and the way in which needs of those concerned with the varied fields of science and technology as well as of the arts and humanities are served.

R. Brightman

Extinct and Vanishing Birds of the World

By James C. Greenway, Jr. (Special Publication No. 13.) Pp. x+518. (New York: American Committee for International Wild Life Protection, 1958.) 5 dollars.

DR. GREENWAY has performed a most estimable service in completing this scholarly and painstaking analysis.

The large-scale exploitation of the landscape of countries colonized by the modern European has

made disastrous inroads into native faunas. The physical removal of great areas of habitat (for example, forests and swamps) and the ravages of introduced rats and cats have been responsible, more often than direct commercial exploitation, pest-control and 'sport', for the decimation of many kinds of animals.

Yet it is gratifying to see from Dr. Greenway's data that the picture is not yet as bleak as one might have thought. For example, the early British settlers freely ravaged the faunas of North America, Australia and New Zealand—yet only one Australian, one New Zealand and two North American 'full' species are known certainly to have disappeared, and only three more species are thought probably to have become extinct in those countries.

Of the thousands of species of modern birds, only 44 are known to have vanished, and a mere 12 probably have done so, although hundreds have become relatively rare and may be in danger. Of those that have gone, almost all were island forms. No less than 12 species—more than one-quarter of total extinctions—have disappeared from islands of the Central and Western Pacific alone. In certain cases at least, the chief factors responsible seem to be the impact of imported eutherians (particularly rats) on highly specialized island (often ground-nesting) natives.

The ecologist and evolutionist, as well as the conservationist, will find a great deal to interest him in this book; and Dr. Greenway is much to be congratulated on its production. A. J. MARSHALL

Georgia Birds

By Thomas D. Burleigh. Pp. xxx+746+48 plates. (Norman, Oklahoma: University of Oklahoma Press, 1958.) 12.50 dollars.

THIS large and handsome volume, published in co-operation with the U.S. Fish and Wildlife Service, is written and illustrated by men who have worked in the field in Georgia. It keeps commendably to the proper purpose of an area study, without attempting to be also a text-book or field-guide for local ornithologists. Thus, the main information for each species relates to its distribution or occurrence in the State, and to its habits as observable there, with a little background on general distribution and on recognition. There are contributed chapters on the history of ornithology in Georgia and on the physiography of the State. The fine colour-plates by G. M. Sutton depict characteristic species in their Georgian habitats; the photographs are primarily of environmental interest; and there are several distribution maps. LANDSBOROUGH THOMSON

British Herbaria

An Index to the Location of Herbaria of British Vascular Plants with Biographical Reference to Their Collectors. Compiled by D. H. Kent, with the assistance of E. B. Bangerter and J. E. Lousley. Pp. 101. (London: The Botanical Society of the British Isles, c/o Dept. of Botany, British Museum (Natural History), 1957.) 20*s*.

THE native British flora, so far as vascular plants are concerned, is relatively poor in number of species but is of particular interest from the geographical position of the British Isles on the north-western margin of Eurasia and because of the diverse ecological conditions within them. Many of the professional and amateur botanists who have studied British plants accumulated herbaria, and these are in many different institutions or in possession of individuals. To trace the whereabouts of some and to ascertain their value is not always easy, and the account compiled by Mr. D. H. Kent, with the assistance of others, will be useful to many who are making detailed researches on various aspects of the British flora. The list of universities, museums, and other institutions possessing herbaria of or with British plants is a long one. It is hoped that proper attention is given to the continued preservation of the specimens. The alphabetical index to collectors is "to indicate where material of individual collectors is represented, and often this is to be found in several herbaria". Obviously it is scarcely possible to compile a complete index of this kind and a great deal of dependence was placed on replies from those in charge of museums and other institutions. Useful information as to size of collections, dates of collectors, etc., is given under many of the names. There are, however, a great many gaps and it may be that the present publication will result in these being largely filled for a new edition.

W. B. TURRILL

Commutative Algebra

Vol. 1. By Prof. Oscar Zariski and Prof. Pierre Samuel with the co-operation of I. S. Cohen. (The University Series in Higher Mathematics.) Pp. xi+29. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1958.) 52*s*. 6*d*.

THE present book makes its appearance after two decades of intensive research, in which the authors themselves have played a leading part. It will be of great value to graduate students and research workers who wish to specialize in this branch of mathematics, for it is the only connected account of the subject. After a chapter of basic definitions and concepts there is a polished account of the theory. This proceeds via field theory and the theory of ideals and modules to the properties of Noetherian rings. The ideal structure of such rings is thoroughly explored. The last chapter is concerned with Dedekind domains and classical ideal theory. Examples illustrating the theory are given in places. But the reader may find the account rather concentrated. If he asks why certain topics are introduced he will find no answer, for it is hidden in the geometric background to the whole theory and there is no hint of this except in the preface, where we are promised the motivation in the second volume. A surprising feature is the apparently complete lack of any references, either to other books or to original papers. It will be difficult for the reader to link the work with the related literature unless he is under expert guidance.

Commutative algebra began as a branch of abstract algebra in Germany and remained a child until the late thirties. Now it is a towering giant (hence this first of a two-volume work), existing in its own right. One questions whether the subject can expect to attain a position of near immortality, at the side of other branches of 'permanent' mathematics. It is true to say that it only grew because the parlous state of the theory of algebraic geometry seemed to point to the need for a new branch of algebra. Now we have it. But on the whole it has not yet done the job of work for which it was introduced. Nowhere in this first volume is there any indication of the motivation behind the development and the reader or prospective reader may, in looking for the purpose, be disappointed and close the book. It is to be hoped that the book has a better reception than this, but the reviewer has his doubts. L. S. GODDARD

ROTATION OF ARTIFICIAL EARTH SATELLITES

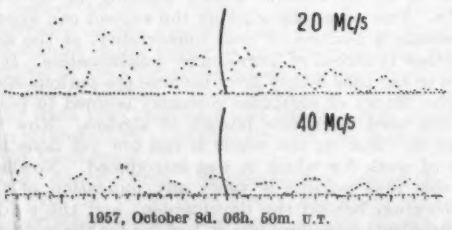
By Prof. R. N. BRACEWELL and O. K. GARRIOTT
Radio Propagation Laboratory, Stanford University, California

A MARKED feature of the signals received from *Sputnik* 1 was the deep, regular fading having a semi-period of about 4 sec. Because of the persistence of this effect from day to day, and because it occurred on both the 20 and the 40 Mc./s. transmission, it seemed likely to be due to free motion of the satellite about its centroid. Simultaneous records exhibiting agreement are shown in Fig. 1. It is true that such simple behaviour was not invariably recorded (Fig. 2); however, if different aerials, or combinations of aerials, were used at the different frequencies, then mechanical rotation of the satellite could modulate the two transmissions in different ways; and further complications could be introduced by the rapidly changing aspect of the satellite and by propagation phenomena. The modulation under discussion is probably ascribable to mechanical rotation, but in any case it is clear that deep fading is to be expected and that it is an embarrassment for telemetry, radio position finding and ionospheric investigations. For example, in Fig. 3, the more rapid fluctuations on 20 Mc./s. contain information about ionospheric ionization[1], but some of the useful detail is obscured by the deep motional fading; and in Fig. 2 the ionospheric phenomenon is entangled with motional effects.

Evidently it is important to know about the state of rotation of satellites, and here means will be considered whereby rotational effects may be eliminated. Fig. 4, which shows a recent field-strength record on *Sputnik* 3, suggests that the problem may already be solved.

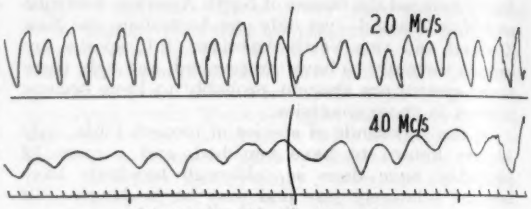
Consider a pencil-shaped satellite, such as *Explorer* 1, which is launched in a state of rotation about its longitudinal axis. At the moment of *Brennschluss* of the final rocket of the final cluster, let the instantaneous axis of rotation make a small angle α with the axis of symmetry of the satellite. Then the angular momentum vector $\vec{\mathbf{h}}$ will be coplanar with the axis of symmetry and the angular velocity vector $\vec{\omega}$, making an angle β with the latter (Fig. 5). The angle β will depend on I and J, the axial and transverse moments of inertia, as follows:

$$\tan(\alpha + \beta) = \frac{\tan \alpha}{m} \qquad (1)$$



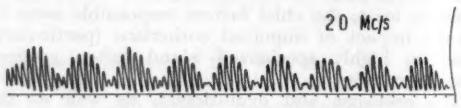
1957, October 8d. 06h. 50m. U.T.

Fig. 1. Simultaneous recordings of the signals received at 20 and 40 Mc./s. showing agreement indicative of satellite rotation. Recorded at Stanford, October 7, 1957, on horizontal dipoles. At this time the transmission was keyed. Time-marks in Figs. 1–4 are at 1-sec. intervals



1957, October 19d. 13h. 14m. U.T.

Fig. 2. Some anomalous observations on 20 and 40 Mc./s.



1957, October 9d. 15h. 13m. U.T.

Fig. 3. Rapid fluctuations associated with ionospheric ionization when the satellite passed over Stanford at great height

where $m = I/J$. For a very long and thin satellite not spinning true ($\tan \alpha \gg m$), the angular momentum vector will be almost perpendicular to the rod. (That this is so is evident from the instantaneous motions of the nose and tail, perpendicular to the paper (Fig. 5), in opposite directions.) For example, launching a homogeneous cylinder 6 ft. long and 6 in. in diameter, only 2° off true, will displace the angular momentum 74°.

The ensuing force-free motion, or Poinsot motion, consists of an axial spin $n = \omega \sin \beta \operatorname{cosec} (\alpha + \beta)$ about the axis of symmetry superimposed on a slewing motion about the invariable line (the direction of total angular momentum) with an angular velocity $\Omega = \omega \sin \alpha \operatorname{cosec} (\alpha + \beta)$. To a rough approximation in the present special case:

$$n \simeq -\omega \cos \alpha$$

and

$$\Omega \simeq \omega \sin \alpha$$

This motion can be visualized as the rolling of a cone of semi-vertical angle α fixed to the satellite, on a fixed cone of semi-vertical angle β.

Thus, in addition to spinning with an angular velocity just below the rate imparted intentionally, the rod would execute a tumbling, waltzing or screwing motion, depending on the orientation of the invariable line to the direction of flight; and since this orientation changes it might waltz overhead at one place and proceed overhead like a propeller at another a quarter of a period later.

Undesirable as this is, both the rate at which it occurs, and the direction of the invariable line, may be set by accidental circumstances of launching not known in advance. It is now very interesting to draw a contrast with the much more favourable phenomena exhibited by a disk.

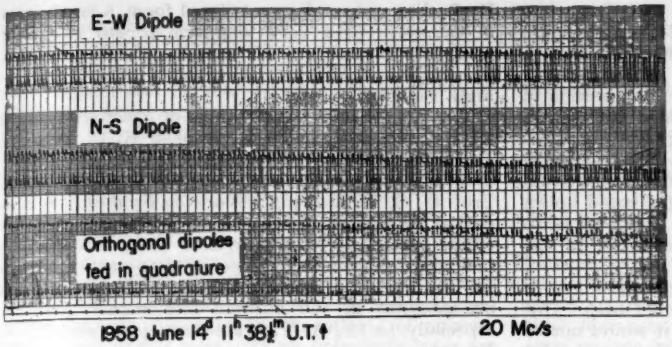


Fig. 4. Field strength record of *Sputnik* 3 which shows Morse-type modulation but which is free from pronounced rotational modulation, thus simplifying the study of interesting effects which shed light on the ionosphere

Let a disk be launched with an angular velocity $\vec{\omega}$ which by accident departs by an angle α from the axis of symmetry. Then the angular momentum $\vec{h}$ falls midway between this axis and $\vec{\omega}$, as may be deduced from equation (1) by putting $m \simeq 2$ (Fig. 6). The disk spins about its axis of symmetry with an angular velocity $n = (m - 1)\ \omega \cos \alpha$ and rocks about the invariable line with an angular velocity $\Omega = \omega \sin \alpha \operatorname{cosec} (\alpha + \beta)$, as before, but in this case the approximations for small α are:

$$n \simeq \omega \cos \alpha$$
$$\Omega \simeq 2\omega$$

Small errors thus produce small effects.

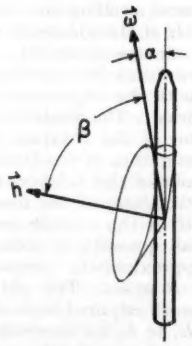


Fig. 5. Relationship of angular momentum $\vec{h}$ to a rod-shaped satellite

If a pair of transmitting dipoles, excited in quadrature, are placed orthogonally in the equatorial plane of a small satellite rotating precisely about its axis of symmetry (Fig. 7), clearly no modulation of signal level would be noticed at any place on the ground (other than small effects due to modification of the dipole radiation pattern by the satellite body). There would simply be a frequency shift equal to the number of revolutions per second. Such an antenna is therefore basically good for counteracting the effect of rotation on field strength and has been described in *Radio*[2]. If now the angle α is not quite zero, then in the case of a disk an observer not near the nulls of the radiation pattern will notice shallow amplitude modulation the rate of which is identifiable as twice the spin frequency. By contrast, the signal from the rod will be deeply modulated, at a rate not necessarily known in advance.

A disk-like satellite ($m \simeq 2$) thus has advantages. It is not necessary that the appearance should be disk-like, only that m be greater than 1. For example, *Sputnik* 3, a conical shell containing batteries fixed in its base[3], may well be disk-like as regards its moments of inertia.

Very interesting questions now arise as to whether the rocking or slewing motions may be suppressed, following the launching. Evidently, one way would be to operate suitable auxiliary rockets. Suppose, however, that the satellite contains viscous fluid or a vibration damper roughly tuned to the rocking frequency, which converts kinetic energy of rotation to heat. Then it can be seen, by bearing in mind the invariance of $\vec{h}$, that in the case of a disk the rocking will be damped out, and that the final angular velocity ω_2 will be given by

$$\omega_2 = \omega\left(\cos^2 \alpha + \frac{\sin^2 \alpha}{m^2}\right)^{\frac{1}{2}}$$

This will leave the angular momentum unchanged but will mean a loss of energy of $\frac{1}{2}J(1 - m^{-1})\,\omega^2 \sin^2\alpha$, or approximately half the energy associated with the rocking motion. In the case of a rod, the spin will be damped out, and the rod will be left slewing exactly in a plane with a final slewing velocity Ω which is unchanged:

$$\Omega = \omega \sin \alpha \operatorname{cosec} (\alpha + \beta) = \omega\,(\sin^2\alpha + m^2\cos^2\alpha)^{\frac{1}{2}}$$

which may be very different from ω. The loss of energy is $\frac{1}{2}\,I\,n^2/(1 - m)$, which is of the same order of magnitude as the spin energy. The possibility of stabilizing a disk by dissipation is another advantage in favour of this shape. Using a vibration damper of quality factor Q capable of storing one Nth of the

Fig. 6. Angular momentum and angular velocity of a disk-shaped satellite

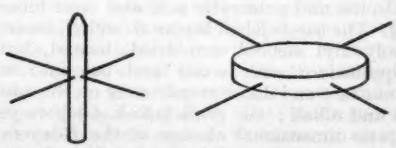


Fig. 7. Dipoles excited in quadrature

energy of the rocking motion, the damping time constant would be QN spin periods, which could readily be made a very small fraction indeed of a satellite's life.

This satisfactory result leaves only the question of also removing the spin rotation, which will, of course, be necessary in experiments requiring instruments to be pointed at celestial or terrestrial objects. Here it appears that the removal of angular momentum would suffice. For example, one might speed up an internal flywheel until the satellite body came to rest, and eject the flywheel together with all the angular momentum.

However, any method of increasing the moment of inertia about the axis of rotation would help. One might re-arrange the contents. Now it would not be necessary to reduce the satellite exactly to rest; for the purpose of observation from the ground one could accept a small rotation during the few minutes that the satellite was above the horizon of a given point, and the same applies to many celestial observations. By feeding out, under centrifugal force, a small mass in the form of a very long fine thread of strong material, the moment of inertia could be increased by a substantial factor and the spin velocity accordingly reduced.

The spontaneous observations of *Sputniks* 1 and 2 were participated in by D. Annett, K. C. Yeh and D. Pratt, members of the Radio Propagation Laboratory, Stanford University, under the direction of Prof. O. G. Villard, jun. Work on *Sputnik* 3 is supported by a grant from the U.S. National Committee of the International Geophysical Year. We are also indebted for stimulating discussion to participants in the Radio Propagation Seminar, which concentrated on satellites in the autumn and winter quarters, especially to Dr. A. T. Waterman, jun., and Mr. J. Fontana.

[1] Munro, G. H., and White, R. B., *Nature*, **181**, 104 (1958).
[2] Vakhnin, V., *Radio*, No. 6, 14 (1957). Kazantsev, A., *Radio*, No. 6, 17 (1957).
[3] *Pravda*, May 18, 1958.

SIZE AND SHAPE CHANGES OF POLYELECTROLYTES: CONVERSION OF CHEMICAL INTO MECHANICAL ENERGY

CONTINUING earlier seminars[1] on polymer science in the Chemistry Department of University College, London, six lectures on size and shape changes of polyelectrolytes and on conversion of chemical into mechanical energy were given on April 14.

Prof. W. Kuhn (Basle) talked on the transformation of chemical energy into mechanical energy by high polymer systems made from vinyl alcohol and allyl alloxan, the alloxan component of which can be catalytically hydrogenated to a dialuric acid component. Slightly cross-linked filaments, containing this copolymer, swollen in dilute acetate buffer contract on hydrogenation and expand on oxidation, due to swelling and deswelling, produced by an increase of solubility in passing from the sodium salt of dialuric acid to alloxan. Thus, mechanical energy is produced from chemical energy arising from the oxidation-reduction process[2,3]. In a previously described system, the chemical energy of neutralization had been transformed into mechanical energy with the use of filaments containing polyvinyl alcohol and polacrylic acid[4], dilation and contraction being brought about by pH changes. In these systems the length changes were accompanied by changes of the lateral dimension. A third system containing alternating layers A and B has been prepared, wherein length changes, exceeding 100 per cent, occur, without alteration of the fibre diameter[5]. A is prepared from heated cross-linked polyvinyl alcohol, and B is prepared from equal parts of polyvinyl alcohol and polacrylic acid also cross-linked by heating. The sandwiched layers A and B, moistened with polyvinyl alcohol, are dried, heated, brought into equilibrium with water and cut into strips. These contract and dilate respectively on the addition of acid and alkali; the cross-linked A-layers permit only a one-dimensional change of the B-layers and thus prevent a change of the lateral fibre dimension. In the dilated system the one-dimensional swelling of the B-layers is accompanied by optical birefringence. Swelling and birefringence disappear on the addition of acid. The volume change of these filaments during contraction or dilation is less than in the three-dimensional swelling and contraction; and the tensile strength of the filaments is maintained if the cross-section remains unaltered. The magnitude of the birefringence and the unidirectional swelling is in accordance with the requirements of the theory of polymer networks[6]. The stretching of the system involves a dilution of the B-layers owing to water uptake. An investigation of the Donnan-equilibrium between the interior of the B-layers and the outside shows that the stretching of the filaments produces an increase of pH in the outside medium. In the presence of a small quantity of sodium chloride the pH change is approximately proportional to the elongation of the B-layers. This pH change is such that the osmotic work required to stretch the B-layers chemically from L_1 to L_2 by increasing the degree of ionization from α_1 to α_2, and subsequently to return at constant lengths L_2 from α_2 to α_1, is equal to the mechanical energy required to stretch the B-layers mechanically at constant value α_1 from L_1 to L_2.

In the discussion, Prof. E. D. Hughes (London) asked whether the rate of the swelling and deswelling reactions is sufficiently large to make it feasible that processes of this kind are responsible for muscular activity. Prof. Kuhn replied that in his systems the rate appears to be diffusion controlled and depends on the fibre diameter. R. E. Cooper (London) referred to a paper by Hill[6], which shows that muscular activity can be so fast that diffusion from the surface of a fibre to its core cannot be rate-determining. If, however, in a twitch the outer region of the fibre were involved (for example, one half of the fibre diameter) then the rate of diffusion

is fast enough to account for the observed rate of fibre contraction. In this connexion, Cooper referred to experiments[7] which showed that the length, the birefringence and the elasticity of fully swollen polyelectrolyte fibres can be markedly altered, at constant *p*H, by stoichiometrically well-defined cation exchange reactions.

Prof. J. A. V. Butler[7a] (London) gave a lecture on effects of salts on configuration of polyelectrolytes in which the electrolyte groups are ionized to a considerable extent. Two classes of linear polymers were considered: (*a*) those with flexible chains; (*b*) those consisting of stiff chains. The configuration as deduced from their viscous behaviour in solution was discussed especially with reference to the effects of concentration and added salts. These polyelectrolytes are very sensitive to added ions, but there are marked differences, in that in (*a*) the effect of ions on the shape of the particle is the predominating factor, while with (*b*) the effect of added ions is principally on the interaction between particles. The behaviour of gels formed from these polyelectrolytes by the introduction of cross-links was discussed.

R. E. Cooper (London) lectured on polymerization of polyelectrolytes by magnesium ions[8]. Alginic acid (poly-*d*-mannuric acid) can be converted into water-soluble potassium, sodium and magnesium salts of well-defined stoichiometric composition. After special purification procedures the weight-average molecular weight of the potassium and sodium salt is below 3×10^5, while that of the magnesium salt is between 10^6 and 10^8. The hydrodynamic and other properties of these polyelectrolytes show that the increase of molecular weight does not give rise to a corresponding increase of molecular volume. It is concluded that the replacement of a monofunctional by a bifunctional counter ion gives rise, at constant *p*H, to a polymerization reaction in which a relatively small polyelectrolyte ion is converted into a larger, but much more compact, species. The effect is explained by the assumption that bivalent magnesium counter ions combine with the carboxyl and hydroxyl groups of the poly-acid to form inter- and intra-molecular bridges. The former are responsible for the large molecular weight, while the latter are responsible for the more compact configuration. Monovalent sodium or potassium counter ions, on the other hand, are not capable of producing cross-links.

In the discussion, Dr. H. Morawetz (Brooklyn) said that his work indicated the role of inter- and intra-molecular cross-links. In his systems molecular aggregation could be followed osmometrically, while group association was obtained from spectroscopy[9]. In studying the interactions with copper[10], the tendency of counter ions to associate with several groups attached to the same polymer could be clearly demonstrated.

According to Cooper and Wassermann[8], the properties of magnesium alginate depend on the sequence of operations in the preparation. Normally, the formation of magnesium chelates with carboxylic acids is rapidly reversible, and Cooper and Wassermann's results must be due to the co-operative effect of many complexes in a molecular aggregate. It has been observed that some polyvinyl chloride solutions attain their equilibrium state rather slowly; and similar effects were noted by Boetger and Doty[11] in gelatine solutions.

Dr. H. E. Huxley (London) talked on structural changes during contraction of striated muscle[12]. The contractile material of striated muscle is composed of longitudinal protein filaments. There are two types of filament present, one type having a diameter (in frog and rabbit muscle) of about 100 A. and the other about 50 A. The thicker filaments are approximately 1·5 mμ long and contain actin. Filaments of each type are arranged in a succession of discrete arrays which overlap with arrays of the other type of filament, the two types of array occurring alternately along the length of the filaments and producing a band-pattern. During contraction and stretch the two arrays slide in or out of each other without any perceptible change in the length of the filaments of which they are composed. This seems to be brought about by cross-bridges between adjacent thin and thick filaments, and which correspond to the sites of actin myosin interaction. The cross-connexions occur at quite short intervals. The process by which chemical energy is transformed into mechanical work does not seem to be associated with appreciable changes in the length of the filaments.

Commenting on this lecture, Prof. W. T. Astbury (Leeds) referred to work[13,14] which shows that the X-ray diagram of dry skeletal muscle is effectively the sum of the diagrams of myosin and *F*-action; and he pointed out that it is difficult at the moment to make the further desirable co-ordination with Huxley's electron microscope findings on what happens during contraction and stretch. Two other fine-structural lines of approach, however, are beginning to run together rather promisingly. In the first place, the concept of muscular contraction as a special manifestation, in myosin, of the property of supercontraction[15], and of the close association of the latter with the cross-β diagram[16], has lately been revitalized by the discovery of natural cross-β forms in bacterial flagella[14] and the egg-stalk of *Chrysopa*[17]. For these and other reasons[18], especially the configurational analogy between the polypeptide chains in the egg-stalk and basal pedestal of *Chrysopa* and the polyethylene chains in the astonishing crystals investigated by Keller[19], there can be little doubt now that the polypeptide chains in myosin, normally in the α-configuration, cannot merely be pulled out reversibly but can be contracted reversibly into a transversely folded configuration. In the second place, there is the recent demonstration by Doty and his collaborators[20] that the make-up of orthodox globular proteins, too, can consist in part of α-helices and that, moreover, the proportions of these can be reversibly varied to some extent. It follows in principle that there must be reversible changes in shape, when protein corpuscles form linear or other aggregates, with their neighbours. This provides the last decisive step in resolving the paradox between the long-range contractile fibre as a straightforward 'molecular yarn' and as a chain of corpuscles[18]. By virtue of these now established intra-corpuscular configurational changes, shortening of the latter can take place either directly or, more generally, by spiralization. Szent-Györgyi's discovery[21] that, by appropriate urea treatment, it is possible to dissociate myosin into small corpuscular units ('proto-myosins') thus becomes not too difficult after all to reconcile with the classical X-ray point of view, and it is consistent also with the inference that bacterial flagella are of the nature of monomolecular muscles[14], and with the ultimate corpuscular construction of algal flagella[14], but unfortunately no approach along lines such as these seems compatible with Huxley's conclusions from electron microscope studies.

Dr. D. R. Wilkie[21a] (London) gave an account of the relationship between thermodynamics and the interpretation of muscle heat measurements. Active muscles produce a relatively large amount of heat, both during activity and during recovery; and the heat production is linked in some way to the mechanical change during contraction. Some of the chemical processes taking place in the muscle have been identified, and several of them are associated with large entropy changes. This introduces a serious uncertainty into the interpretation of the heat measurements.

C. A. Vernon (London) talked about the concept of phosphate bond energy[22]. The term 'high-energy phosphate bond' was first introduced by Lipmann[22] and had reference to the fact that the standard free energy of hydrolysis of adenosine triphosphate (ATP), which is said to possess a high-energy phosphate bond, is considerably higher than that of most simple orthophosphate esters.

Lipmann's concept has, however, led to considerable confusion and readily gives rise to pseudo-explanations concerning the role of adenosine triphosphate in biological processes. The quantities which provide the experimental basis for the high-energy phosphate bond concept are not bond energies, but standard free energy changes. As such they may may be used to calculate equilibrium constants, or to specify maximum work at unit activities. They do not provide explanations for the role of particular phosphate esters and are better not described by a highly misleading terminology.

[1] *Nature*, **180**, 535 (1957).
[2] Ramel, Diss. Basel (1957).
[3] Kuhn, *Angew. Chem.*, **70**, 58 (1958). Kuhn, Ramel and Walter, *Chimica*, **12**, 123 (1958).
[4] Kuhn, Hargitay, Katchalsky and Eisenberg, *Nature*, **165**, 515 (1950). Kuhn and Hargitay, *Exper.*, **7**, 1 (1951).
[5] Kuhn, *Kolloid Z.*, **76**, 258 (1936). Kuhn and Gruen, *Kolloid Z.*, **101**, 249 (1942). Kuhn, Pasternak and Kuhn, *Helv. Chim. Acta*, **30**, 1705 (1947); **31**, 340 (1948).
[6] *Proc. Roy. Soc.*, B, **135**, 446 (1948).
[7] Mongar and Wassermann, *Nature*, **159**, 746 (1947), and subsequent papers.
[7a] Cf. Butler, Robins and Shooter, *Proc. Roy. Soc.*, A, **241**, 299 (1957). Butler and Conway, *J. Polymer Sci.*, **11**, 277 (1953). *La Ricerca, Scientifica*, **25** (1955). Conway, *J. Polymer Sci.*, **18**, 237 (1955).
[8] Cooper and Wassermann, *Nature*, **180**, 1072 (1957).
[9] Chang and Morawetz, *J. Phys. Chem.*, **60**, 782 (1956).
[10] Kotliav and Morawetz, *J. Amer. Chem. Soc.*, **77**, 3692 (1955). Morawetz and Sammak, *J. Phys. Chem.*, **61**, 1357 (1957).
[11] *J. Amer. Chem. Soc.*, **78**, 4267 (1956).
[12] Huxley, *Proc. Roy. Soc.*, B, **141**, 59 (1953). *Biochim. Biophys. Acta*, **12**, 387 (1953). Hansch and Huxley, *Nature*, **172**, 530 (1953); **173**, 973 (1954). Hansom and Huxley, Symp. Soc. Exp. Biol., **9**, 228 (1955); *Biochim. Biophys. Acta*, **23**, 229, 250 (1957). Huxley, J., *Biophys. Biochem. Cytol.*, **3**, 631 (1957).
[13] Astbury, *Nature*, **160**, 388 (1947).
[14] Astbury, Beighton and Weibull, Symp. Soc. Exptl. Biol., **9**, 282 (1955).
[15] Astbury, *Proc. Roy. Soc.*, B, **134**, 303 (1947). (Croonian Lecture, 1945.)
[16] Rudall, Symp. on Fibrous Proteins, *J. Soc. Dyers and Colourists*, 15 (1946): "Adv. Protein Chem.", **7**, 255 (1952).
[17] Parker and Rudall, *Nature*, **179**, 905 (1957).
[18] See Astbury, Introduction to section on Macromolecules, Faraday Society's General Discussion on "Configurations and Interactions of Macromolecules and Liquid Crystals" (Leeds, in the press).
[19] Keller, *Phil. Mag.*, **2**, 1171 (1957). Keller and O'Connor, *Nature*, **180**, 1289 (1957).
[20] See, for example, Astbury, *Nature*, **181**, 94 (1958).
[21] Szent-Györgyi and Borbiro, *Arch. Biochem. Biophys.*, **60**, 180 (1956). Szent-Györgyi, *Science*, **124**, 873 (1956).
[21a] "Progress Biophys.", **4**, 303 (1954).
[22] Gillespie, Maw and Vernon, *Nature*, **171**, 1147 (1953).
[22a] "Adv. Enzymol.", **1**, 99 (1941).

ARCHITECTURE OF A PROTEIN MOLECULE*

By Dr. J. C. KENDREW

NEARLY all the molecules most characteristic of living matter are very large ones, containing hundreds or thousands of atoms; proteins, fats, carbohydrates and nucleic acids are typical examples. Living organisms nearly always have well-defined size and morphology, and it is not surprising that they should be built up from units which are themselves large and complicated; furthermore, the subtlety of the chemical reactions carried out by cells demands a corresponding complexity in the molecules which mediate them.

Among these biological macromolecules the proteins are paramount. Some proteins are used as structural components, for example, the long filamentous protein molecules of bone, of tendon, of hair, of skin; others compose the muscle fibres which convert chemical energy derived from food into the mechanical energy of a moving limb. Another kind of protein molecule are the so-called enzymes, the essential catalysts which control the rate and determine the ordered sequence of the chemical reactions enabling each living cell to play its proper part in the complex economy of the organism, in a manner quite beyond the reach of the chemist in his laboratory. The enzymes are perhaps the most interesting of all types of protein, and they are members of the class known as globular proteins, so named because their molecules are nearly spherical.

Large molecules found in living organisms are generally built up by the combination of a great number of small molecules of a few standard types. For example, nucleic acid molecules are made of only four types of standard part—the nucleotides—arranged in linear sequence along a chain, the difference between individual nucleic acids residing in the difference of order of the units, just as the meaning of a message in the Morse code is conveyed by its sequence of dots and dashes. In proteins the standard parts are called amino-acids; and although hundreds or even thousands of amino-acids, arranged linearly in chains, may be contained in a single protein molecule, these amino-acids are of only twenty different kinds. In the proteins the analogy is to written language rather than to the Morse code; we may think that they differ in behaviour in consequence of their differing amino-acid sequences in much the same way as the meaning of a sentence depends on the order of the letters of which it is composed. Each kind of amino-acid consists of the same standard 'link' (the chemical group $NH_2.\underset{|}{C}H.COOH$) attached to a 'side-chain' characteristic of the par-

* Based on an Evening Discourse delivered at the Royal Institution on May 21.

Fig. 1. The α-helix of Pauling and Corey. The side-chains of the amino-acids are all denoted by *R*

ticular amino-acid. When joined together the links form the so-called backbone (or main chain), from which project side-chains of twenty different kinds; the whole is called a polypeptide chain.

The actual sequence of the amino-acids in a protein was first determined by Sanger in the case of insulin. Sanger showed that every insulin molecule from a given species of animal contained the same fifty-one amino-acid residues arranged in the same order, and that the insulins of different species were nearly identical, differing by only one or two amino-acids in the whole molecule. In myoglobin, the protein which forms the subject of this article, there are more than 2,500 atoms, comprising 153 amino-acids linked together in a single chain. The amino-acid sequence of a protein is called its primary structure, and its determination for insulin was a great triumph of structural chemistry. But it is not the whole story: since the globular proteins are nearly spherical the amino-acid chains of which they are composed must be folded and re-folded like a ball of string. Furthermore, many proteins form very perfect crystals, which means that their molecules must be identical in shape as well as in amino-acid sequence, otherwise they could not pack together in the highly regular manner which a crystalline structure implies. We must therefore address ourselves to the question how the amino-acid chain in a protein is folded—for it is evident that the chemical behaviour of the complete molecule will depend on the spatial relationships of nearby amino-acids in the folded structure.

In 1951 Pauling and Corey showed that in some of the filamentous proteins of hair and skin and wool the polypeptide chain is arranged in the form of a regular helix, known as the α-helix (see Fig. 1); this is the so-called secondary structure of these proteins, and its presence is revealed by the X-ray pattern of the fibre, first studied in detail by Astbury in 1933. The α-helix has been shown to be present in many protein fibres, and there is suggestive evidence (though as yet no proof) that it also exists in globular protein molecules. By its nature, however, the α-helix is rigid and straight; a molecule made up of the 153 amino-acids in myoglobin, all in the form of a single α-helix, would be twenty times as long as it was wide, whereas, in fact, myoglobin is nearly spherical. It follows that there must be a yet higher category of fold—a tertiary structure—to bend the helix within the compass of a spherical molecule. Even this does not exhaust the complexities of protein molecules, for they often contain, in addition to amino-acids, some other type of chemical group, the so-called prosthetic group. Myoglobin contains such a group, a flat symmetrical arrangement of atoms with a single iron atom at its centre, known as the hæm group: it confers a red colour on myoglobin. When associated with the protein the hæm group has the property of combining reversibly with a molecule of oxygen; and indeed the function of myoglobin is to act as a temporary store of oxygen in the muscle cells, just as that of hæmoglobin (which also contains hæm groups) is to transport oxygen in the blood stream. Diving animals such as whales, seals and penguins need to store oxygen for long periods while they are under water, and accordingly contain large amounts of myoglobin so that their meat is very dark red; thus the studies of myoglobin about to be described were done with material derived from the sperm whale.

The only way of finding out the spatial arrangement of the atoms in a complex molecule is by studying the X-ray diffraction pattern of its crystals. The first structure so to be determined was that of sodium chloride. This molecule contains only two sorts of atom, and since 1913, when its structure was worked out by Sir Lawrence Bragg, the method has developed far, its most recent triumph being the determination of the complete structure of vitamin B_{12} by Dr. Dorothy Hodgkin. There are 93 atoms in vitamin B_{12} (excluding hydrogen, which is so light that its position cannot be found by ordinary X-ray methods); but even a small protein molecule like myoglobin contains more than ten times as many atoms as does the molecule of vitamin B_{12}, and it is simply beyond the scope of the method in its present state of development to contemplate determining the complete structure of a molecule as complicated as this, in the sense of discovering the exact relative positions of all its atoms. All that can at present be hoped for is to find out something about the general layout of the molecule—its tertiary structure, in fact.

One may regard each spot in the diffraction pattern of a crystal as corresponding to a train of waves running through the crystal. If all the waves are added together the result is a representation of the distribution of matter throughout the repeating unit (or unit cell) of the crystal, just as the wave-form of a musical note can be reconstituted by adding together the harmonics into which it may be analysed. If the waves are to be correctly combined, it is necessary to know four things about each: its direction, its wave-length, its amplitude, and its phase. The first three can be determined immediately by measuring the X-ray diffraction pattern; but the phase—the amount by which each wave is out of step with its neighbours—cannot be discovered directly by any physical method. This is the so-called phase problem of X-ray crystallography—if the phases could be found by direct measurement, X-ray structure determination would simply be hard work: as it is, cunning is needed as well as industry.

Simple structures have often been worked out by the trial-and-error method, which in essence consists in making intelligent guesses on the basis of previous knowledge about the molecule being studied. In

proteins, however, the structure is too complicated for anyone to be able to make such guesses. Since the first X-ray pictures of protein crystals were taken by Bernal and Crowfoot in 1934, a large number of theories of protein structure has been based on them, but these were all speculative and, as it has turned out, much over-simplified. Speculation gave place to hard fact only as a result of the discovery, by Perutz in 1953, that it was possible to apply to protein crystals the so-called method of isomorphous replacement, which had often been applied to simpler structures, but which no one had, until then, shown could be used to study anything as complicated as a protein. The essence of this method is to add to each protein molecule in the crystal a single very heavy atom, such as mercury or gold; this has the effect of slightly changing the intensities of most of the spots in the diffraction pattern. It turns out that, by comparing the diffraction patterns of two crystals which differ only in the inclusion of the heavy atom in one of them, it is possible to solve the phase problem by an unambiguous procedure which does not involve guesswork.

Perutz applied this method to hæmoglobin, and by its use he was able to obtain a two-dimensional projection of the structure, as it were a silhouette of the molecule. This projection was derived by straightforward deductive methods, and it is certainly correct; but, unfortunately, the two-dimensional silhouette is so confused owing to overlapping that one can learn from it virtually nothing of the spatial arrangement of the atoms composing the molecule. In hæmoglobin one is actually 'looking through' the thickness of some forty atoms, so it is not surprising that the projection is uninterpretable. We subsequently applied the same method to myoglobin, the molecule of which is only one-quarter the size of hæmoglobin, and derived a similar two-dimensional projection which proved to be equally obscure.

It was clear that the only way to make progress was to extend the method to three dimensions. Such an extension is not easy, for several reasons. First, in order to get an unambiguous result in three dimensions it is necessary to compare the diffraction patterns of several crystals, each containing a heavy atom in a different place; secondly, the computations involved in determining the phase of each reflexion are more tedious than in two dimensions; and thirdly, for any desired resolving power it is necessary to examine far more reflexions.

In our recent studies on myoglobin we were able to attach four different heavy atoms at different places on the molecule, and to compare the diffraction patterns of each of the resulting substituted crystals with that of the protein alone. As to resolving power, the number of reflexions which must be included in the calculations in three dimensions is proportional to the cube of the resolution desired. Individual atoms are 1–2 Ångström units apart, and to see them separately would demand a resolution of at least 1·5 A., involving the measurement of more than 20,000 reflexions per crystal. Our plan was to try out the method, and to gain a first look at the structure, at low resolution. We chose the figure of 6 A., because at this resolution one might expect to see the polypeptide chains if they were in some compact configuration such as the α-helix: to attain this resolution it is only necessary to study 400 reflexions from each crystal.

The work of measuring these reflexions for crystals of myoglobin and of each of five different substituted myoglobins was carried out in the Medical Research Council Unit for Molecular Biology at the Cavendish Laboratory, Cambridge, and at the Davy Faraday Laboratory of the Royal Institution, in collaboration with Drs. Bluhm, Bodo, Dintzis, Phillips, Wyckoff and Miss Mary Pinkerton. After completing the calculations needed to determine the phases of all the four hundred reflexions, we computed an electron density map on the electronic computer EDSAC at Cambridge; this calculation was checked by Dr. Rollett, who repeated the computation on DEUCE at the National Physical Laboratory. The result was plotted in the form of sixteen sections through the unit cell, piled up (like the serial sections of organs used by anatomists) to give a three-dimensional representation of the density of matter throughout the unit cell, the density at each point being represented by contours (Fig. 2).

It was gratifying to discover that in the resulting three-dimensional synthesis there were indeed numerous rod-like features of just the dimensions and density to be expected of polypeptide chains arranged in a helical configuration. It is not easy to see these rods except by looking at the synthesis itself, but some of them are visible in the right-hand half of Fig. 2. There is little doubt that these rods are actually blurred representations of the polypeptide chains, and by adding up all the visible lengths of rod we can account for some two-thirds of the whole molecule.

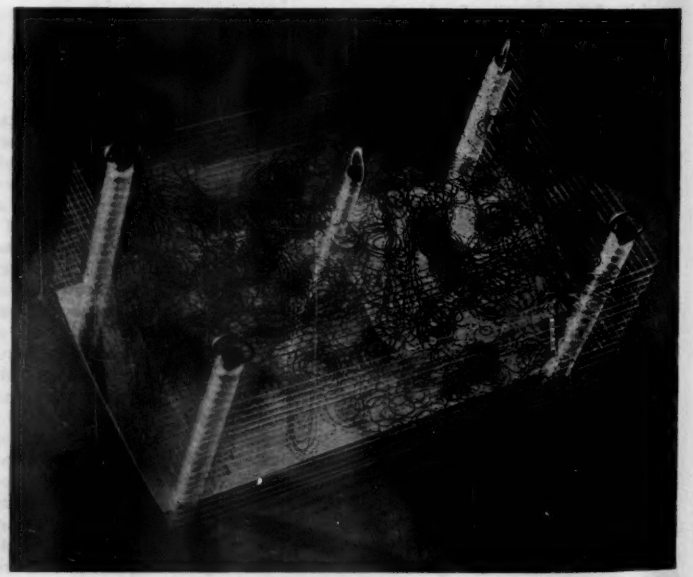


Fig. 2. Photograph of the three-dimensional electron density map of the unit cell of myoglobin. Two polypeptide chains can be seen on the right: the dense peak between them is the hæm group

Fig. 3. Photographs of a model of the myoglobin molecule. The marks on the scale are 1 A. apart. The grey disk is the hæm group; the pair of small spheres attached to it is an oxygen molecule on the same scale. The larger spheres are heavy atoms used to solve the crystal structure

Another prominent feature of the synthesis is a flattened disk-like region the density of which exceeds the mean by some 50 per cent more than does that of any other part of the map. We think that this region is the hæm group itself, the high density being contributed by its central iron atom, the heaviest atom in the myoglobin molecule. The identification is confirmed by the orientation of the disk relative to the axes of the unit cell, which corresponds with the hæm group orientation deduced from a study of the optical and magnetic properties of the crystal; and also by the fact that one of the heavy atoms we were able to attach to myoglobin, the iodine atom in *p*-iodophenylhydroxylamine, which is known to be a specific reagent for the hæm group itself, has been located in the immediate neighbourhood of the disk.

The unit cell of myoglobin actually contains two molecules of protein, related by a symmetry axis (a screw dyad), and the electron density map likewise contains two molecules, each with its own hæm group and polypeptide chains. Furthermore, the two molecules in a single unit cell are closely surrounded by the molecules of neighbouring unit cells. To get an idea of the structure of a single myoglobin molecule one must, as it were, dissect out a single molecule from its neighbours. To do this, we make use of the fact that a protein crystal generally contains up to 50 per cent of liquid, which fills up the interstices between the protein molecules. In the electron density map we can recognize these interstices, because the liquid is featureless and has uniform electron density. Once the interstices have been located, the boundaries of a single molecule can be outlined in a straightforward manner.

Fig. 3 shows two photographs of a model of the single molecule thus separated from its neighbours. The sinuous white rods are the backbone of the polypeptide chain: it must be remembered that in reality these backbones are clothed with a layer of side-chains (invisible at this resolution) so thick that each polypeptide chain is in contact with its neighbours. Also marked is the hæm group, a flat grey disk; and on it are two spheres which represent an oxygen molecule and which have been included to convey some idea of the scale of the model, although, of course, they cannot actually be seen in the electron density map. The model also shows some of the heavy atoms which were attached to the molecule in order to solve the phase problem.

Chemical evidence indicates that myoglobin consists of just one single polypeptide chain, made up of 153 amino-acid residues. In the model this chain pursues a complicated winding path throughout the molecule, which has overall dimensions about $43 \times 35 \times 25$ A. It may be asked whether it is possible to trace the course of the chain unambiguously through the molecule: in most places this can be done, but in some, for example, where it turns through a particularly sharp corner, the chain fades out at the resolution used in this map, and one cannot be sure of its course. It is clear that whenever the chain turns a corner it must lose its helical configuration for, as has already been pointed out, an arrangement such as the α-helix is essentially straight and cannot bend without ceasing in effect to be a helix at all: at the resolution of 6 A. an extended polypeptide chain would scarcely be visible. Measurement of the total length of chain in the model suggests that it is a helix for some 70 per cent of its length, a proportion which agrees well with estimates based on other techniques.

All in all, the model poses more questions than it answers. It gives us a first glimpse of the configuration of a protein molecule, but it is a tantalizing glimpse because the picture is so blurred that one cannot recognize side-chains, nor be certain how the backbone is folded—even whether it is a helix at all—nor know how the hæm group is attached to the rest of the molecule. Many of these questions could be cleared up if an electron density map could be drawn at higher resolution, even if that resolution stopped short of separating individual atoms. There seems no reason in principle why the resolution should not be improved, although an extension to atomic resolution is at the moment beyond our reach. At present, data are being collected for the 10,000 reflexions out to 2 A., and we hope that the electron density map prepared from these will in due course show us some of the finer details of the structure.

NEWS and VIEWS

Ministry of Supply: Scientific Research (Electronics and Guided Weapons): Dr. E. V. D. Glazier

Dr. E. V. D. Glazier, who holds the post of director of Scientific Research (Electronics and Guided Weapons) in the Ministry of Supply, has been promoted to the rank of deputy chief scientific officer. Dr. Glazier, who was born in 1912, had his early training in general electrical and mechanical engineering in industry, joining the Engineer in Chief's Office of the General Post Office in 1933, when for the ensuing nine years he was engaged on the development of telephone systems. He graduated in the University of London and was awarded the degree of Ph.D. In 1942, he transferred under war-time arrangements to the Signals Research and Development Establishment, joining the Line Communication Group where he was responsible for the development of a wide range of signals equipment for the Army. He became head of the Line Communication Group in 1946, having in the interim spent two years in the Ministry of Supply headquarters. In 1950, he was transferred to lead the Research Division at Signals Research and Development Establishment and under his leadership the research group there made significant advances in a number of radio and electronic systems for Army use. In 1957, he returned to headquarters so that his practical training and research knowledge could be employed in advising on and co-ordinating the present and future research in electronics in the Ministry of Supply as a whole. Dr. Glazier is the secretary of the Radar and Signals Board of the Scientific Advisory Council. He is one of a band of people who, having moved progressively from application to research, are of very great value in the senior ranks of the Ministry of Supply.

Ministry of Supply: Electronics Research and Development: Dr. J. S. McPetrie

Dr. J. S. McPetrie has been appointed director general of Electronics Research and Development in succession to Dr. D. H. Black (see *Nature*, 182, 225; 1958). Dr. McPetrie has had a long and distinguished career in radio research. He joined the Radio Research Station of the National Physical Laboratory from the University of Aberdeen, and was engaged on early research on aerials and propagation at very high frequencies. He also explored methods of generating the progressively shorter wave-lengths required for this work. He was the author of several early papers on the properties of metric waves. During the Second World War he joined the Ministry of Supply and was lent to the British Commonwealth Scientific Office in Washington. At the end of the War he returned to take charge of the newly formed Research Division at the Signals Research and Development Establishment, Christchurch, where he was responsible for a wide range of research problems in radio and electronics for the Army. In 1950, he was promoted to be in charge of the Radio Department at the Royal Aircraft Establishment, where he was responsible for research and development in aircraft radio and navigational systems.

Dr. McPetrie has been a leading member of the Institution of Electrical Engineers for more than 25 years, and he was honoured recently by election as chairman of the Radio Section for the session 1957–58. His year of office was notable for the number of highly successful meetings and international conferences that were organized. In his new appointment Dr. McPetrie will be responsible for research and development in communications and radar for the Naval Air Branch, Army and the Royal Air Force, and for many of the other fields in which electronic devices are contributing to defence and civil aviation.

British Scientific Attaché in Moscow

The Department of Scientific and Industrial Research has announced that a senior principal scientific officer is to be appointed scientific attaché to the British Embassy in Moscow (see *Nature*, 182, 292; 1958). There are at present only four British scientific attachés, namely those attached to H.M. Embassies at Washington, Paris and Bonn, with one scientific attaché attached to the Embassies at Stockholm, Oslo and Copenhagen, and working between them. Their duties include reporting on scientific developments in the countries in which they serve and advising the ambassador on scientific questions.

Science Laboratories in Schools

A table circulated by the Parliamentary Secretary to the Ministry of Education, in reply to a question regarding part-time day release in industry, shows that the percentage of boys under eighteen released to attend part-time day classes in 1956–57 has risen to 33·8 (from 27 per cent) and of girls to 8·5 (from 7 per cent). On July 24 Sir Edward Boyle gave the estimated capital cost of new science laboratories started in 1957–58 at schools maintained by local education authorities as £1,100,000 and of those due to start in 1958–59 as £2 million; of these figures £350,000 and £550,000, respectively, were at grammar schools; £600,000 and £1,200,000 at modern schools; and £150,000 and £250,000 at technical secondary schools. Questioned further on July 31, Sir Edward said that extensions to science accommodation at more than 100 grammar schools had been approved for the 1958–59 and 1959–60 building programmes. Although a recent survey reported in the *School Science Review* indicated that less than 10 per cent of maintained grammar schools had reported adequate science laboratories, 44 per cent of the schools had science accommodation conforming with the suggestion made in the Ministry's building bulletin.

Préhistoire Spéléologie Ariègeoises

The activities of the Société Préhistorique de l'Ariège are not as well known as they should be. The first bulletin for 1946 appeared in 1948 and the present one under review is No. 12 (pp. 152+7 planches. Tarascon-sur-Ariège: M. Romain Robert, Place Jean-Jaurès, 1957). One of the better accounts of the great painted cave of Niaux from the pen of Abbé Breuil will be found in No. 5. Each number has been full of interesting articles. The present number is no exception, and it contains many good papers: the rhinoceros and the cave bear as depicted in upper palæolithic art; aurignacian art in central Europe; notes on the Neolithic finds in the Department of the Aude; several shorter accounts of various activities of the Society. Publications by local societies tend to be overlooked and are not always easy to obtain. But prehistorians would be wise to keep in mind the activities of this flourishing society in the Ariège and their energetic president, Monsieur Robert.

Journal of the Institute of Wood Science

In 1957 the Institute of Wood Science was inaugurated for the advancement of timber research and technology, and the first number of the Society's journal has now been published (*Journal of the Institute of Wood Science*, No. 1, March 1958. Pp. i+64. 15*s*.). Its scope is to include original papers, both in research and in technology, and reports of lectures and discussions at the Society's meetings. Research on timber is a very wide subject and involves many scientific disciplines, biological, physical, chemical and engineering, and the new journal should have a varied and interesting range of papers to publish. This is reflected in the first number, the contents of which includes such diverse subjects as variations in structure occurring during growth, the physiology of cambial activity, ultrasonic-pulse technique in investigations of strength, and the acidity of wood. All, as would be expected, have practical application in view, so that the journal should have appeal both for scientific and technological workers alike.

A New Estonian Natural History Journal

The Academy of Sciences of the Estonian Soviet Socialist Republic has produced a new journal of science, *Eesti Loodus* (Estonian Nature). Published bi-monthly, the articles are written in Estonian; but each issue contains abstracts in the English language which have been prepared by a translator with a remarkable flair for the right word and phrase. The articles cover a wide range of subjects including such topics as the artificial earth satellite; cliffs of the Jaagarahu Strata; Virginia witch-hazel in the Estonian S.S.R.; insects on snow; the practical application of the infra-red quenching of luminescence; the dependence of the external form of tetracorals on environmental conditions; the distribution of steppe plants and phytogeographical boundaries in Estonia; the 'Snowman'; ball lightning; the Elva Sandr, its formation and early hydrography; plant parasitic nematodes in Estonia and the protection of inanimate nature in the Estonian S.S.R. Each number also contains a section giving details of outstanding scientific discoveries as well as popular sections for young people and general readers. The editorial office is 5 Hariduse Street, Tartu, Estonian S.S.R.

The Geological Museum

The Geological Museum at South Kensington has just issued an attractive short guide to the exhibits (pp. ii+32. London: H.M. Stationery Office, 1958. 1*s*. 6*d*.). The publication is intended primarily for the visitor who wishes to make a short tour of the Museum and obtain a general idea of the scope of the display collections. At the outset is given a short history of the Museum and a sketch plan of the general arrangement. The three floors are then described in more detail again, with plans of particular sections. The general arrangement of the guide thus conforms with previous editions. The format is good and pleasing, though it is unfortunate that the method of reproduction of the photographic illustrations scarcely does justice to the originals.

The Wild-Fowl Trust

Besides a wealth of characteristic sketches by the Director, the ninth annual report of the Wild-Fowl Trust contains a remarkable collection of photographs of wild-fowl taken by some of the world's best natural history photographers (edited by Peter Scott and Hugh Boyd. London: Country Life Limited, 1958. 10*s*. net). Then there is a series of articles by members of the staff and friends of the Trust which not only records developments and researches at Slimbridge but also describes wildfowl collections and investigations in many other parts of the world. Probably the biggest event in the life of the Trust during 1956–57 was the opening of the Peakirk Waterfowl Gardens by H.R.H. the Duke of Gloucester on April 12, 1957. One of the main objects of this scheme is to provide sufficient funds to enable the Trust to take on the lease and maintenance of Borough Fen Decoy, which has been operated by members of the Williams family since its construction in about 1640. It is also of great value to have a place which can be used as a reservoir for birds, and an alternative collection, as the danger of epidemics among the birds at Slimbridge cannot be entirely dismissed.

During the period popular interest in the collection at Slimbridge continued to grow in a gratifying way. The total number of visitors in the calendar year 1956 was about 120,000. In 1957 this figure was reached before the end of August. Membership stood at 5,094 in January 1957—another record figure. The collections at Slimbridge and Peakirk now contain about 1,747 birds of 148 forms (116 species). In 1957, 770 young birds, of 72 forms, were reared in the collection. Extensive studies have begun in two fields new to the Trust's research programme: a systematic investigation into the food of wild fowl and the use of aircraft in surveys of the distribution and number of wildfowl.

Demonstration Meeting on Biological Acoustics

During the fifteenth International Congress of Zoology, held in London during July 16–23, the Committee for Biological Acoustics organized a demonstration meeting to illustrate the wide range of research in this comparatively new and expanding field. The meeting, held on July 21 in the Imperial College of Science and Technology, London, was open to all members of the Congress and attracted more than one hundred visitors. Work on the reception of human speech was shown by Dr. E. C. Cherry and colleagues of the Department of Electrical Engineering, Imperial College, and demonstrated how recognition of electronically 'clipped' speech is accomplished. Binaural location of sounds was also shown. The progress made in the United States in underwater recording was emphasized by a demonstration of the acoustic emission of whales by Dr. W. E. Schevill, of the Oceanographic Institution, Woods Hole, Massachusetts. The technical problems inherent in such studies were made apparent by recordings of American marine noises made by Dr. Martin Johnson, of the Scripps Institution of Oceanography, University of California; a high-level background noise interfering with recording of fish noises was eventually traced by him to its origin in large populations of 'snapping shrimps'. The single, irregular cracking noise produced by each individual shrimp drifting in the sea, when multiplied by the number of shrimps within range of the microphone, produces a frizzing background noise of high level. British interest in underwater acoustics was underlined by Mr. N. B. Marshall, of the Department of Zoology, British Museum (Natural History), who said that it was hoped that a start would soon be made on recording in the English Channel.

Bird sounds were introduced by Dr. R. G. Busnel, director of the Laboratoire de Physiologie Acoustique, France, who, in collaboration with Prof. H. Frings, of Pennsylvania State University, has recorded the songs of French and American crows to see if their sound signals were similar, or if they had evolved separate 'dialects' of their own. Dr. J. H. Elgood, Biology Department, Ibadan, Nigeria, played some recordings of the songs of Nigerian birds in the wild. Insect acoustics was represented by several workers. Dr. J. W. S. Pringle, Zoological Laboratory, Cambridge, played recordings made by him in Ceylon of the songs of several species of cicadas, and Mr. E. F. Woods, of the B.B.C., demonstrated his work on the sounds of colonies of honey bees, while developing an electronic device, the 'Apidictor', which can assist apiarists in detecting the onset of swarming in hives. Mme. G. Richard, Laboratoire de Zoologie, Rennes, demonstrated recordings of the underwater noises made by corixids, while Dr. W. Loher, Department of Zoology, Imperial College, and Dr. P. T. Haskell, Anti-Locust Research Centre, London, showed some results of their investigations on the stridulation of grasshoppers. Much of the discussion, both public and private, which developed out of the demonstrations was concerned with techniques and instrumentation, and it was therefore helpful that a number of firms, including Ferrograph, E.M.I. and Grundig, had lent apparatus for demonstration.

Time Rates of Wages and Hours of Labour

The twelfth edition of "Time Rates of Wages and Hours of Labour", compiled by the Ministry of Labour and National Service (London: H.M. Stationery Office, 1958. 15*s.* net), contains the usual tables which show, for the majority of industries and occupations, the minimum or standard time-rates of wages, the dates from which these rates become operative, and the normal hours of labour. The rates of wages and hours of labour are those determined by voluntary agreements between organizations of employers and workpeople, or made by Joint Industrial Councils or other similar bodies, or by Statutory Orders under the Wages Councils Acts, the Agricultural Wages Acts and the Catering Wages Act. Particulars are also given, where available, of the basic rates for pieceworkers, the additional rates payable to shift and night workers, and, where they are known to exist, of the arrangements for a guaranteed weekly wage or period of employment. Three appendixes give details of rates of wages for young workers in a selection of industries, and of overtime rates of pay, and arrangements for paid holidays in the industries included in the main part of the volume.

Scientific Film Association

The sixth annual report of the Council of the Scientific Film Association, for the year ended March 31, 1958 (pp. 12. London: Scientific Film Association, 1958), records an increase in membership from 324 to 404 during the year, and the six issues of the *Scientific Film Review* provided technical and distribution details on 335 films; similar information and a synopsis of content for a further 324 films; and full reviews or appraisals of another 451 films. Three catalogues were published during the year with detailed reviews and appraisals of 44 films on cerebral palsy, 100 films on education and 131 films on psychology and psychiatry. The film appraisal work is under continual development and a report on "The Evaluation of Scientific, Industrial and Medical Films" previously published in the *Scientific Film Review* will shortly be re-issued as a pamphlet. The Association has dealt with a wide range of inquiries about films and film making, and besides arranging a varied programme of meetings, held a residential course at Kingsgate College in March on the use of films in supervisory training. As the national representative, it plays an active part in the work of the International Scientific Film Association.

Semi-conductors

A valuable guide to the use of semi-conductor devices, compiled by a committee consisting of representatives of fifteen manufacturers of semi-conductor devices in the United Kingdom, has recently been published on behalf of the manufacturers by the British Radio Valve Manufacturers' Association (The Use of Semi-conductor Devices. Pp. iv+36. London: British Radio Valve Manufacturers' Association, 1958). It is intended to assist equipment designers to secure optimum performance and life from the devices, which consist basically of monocrystalline material. In the case of diodes and transistors the substances are either germanium or silicon, and in the case of photosensitive devices they include also compound semi-conductors such as lead selenide or indium antimonide. The booklet is divided into four sections dealing with, respectively, general considerations, for example, frequency limitations, ratings, temperature and high-energy effects, of semi-conductor devices; semi-conductor diodes; junction transistors; and photo-sensitive devices. The information given is very carefully and clearly set out. A detailed index allows easy and quick reference to any particular item discussed in the guide.

Low-Temperature Thermometer

Emphasis on low-temperature research, particularly low-temperature calorimetry, has highlighted the need for a thermometer which indicates low temperatures accurately and reliably and does not need continual calibration. Germanium can be doped with arsenic to produce a high and fairly constant temperature coefficient at temperatures near to the boiling point of helium, and a germanium resistance thermometer of high sensitivity and exceptional stability in the temperature range near to the absolute zero has been developed by J. E. Kunzler, T. H. Geballe and G. W. Hull of Bell Telephone Laboratories Ltd. The thermometer consists of a single crystal of arsenic-doped germanium, $0{\cdot}025 \times 0{\cdot}020 \times 0{\cdot}210$ inches, with attached current and potential leads and supported in a strain-free manner in a platinum-glass enclosure filled with a small amount of helium. Temperature is determined by measuring the potential drop due to resistance when a current of approximately 10^{-7} amp. is passed through the germanium crystal. A typical thermometer had a resistance of one ohm at room temperature, 14 ohms at 10° K. and 216 ohms at 2° K., but both the temperature coefficient and the actual resistance vary widely with minute changes in the amount of doping, so that thermometers of differing characteristics can be made. Once calibrated the thermometer is reproducible to better than a few ten-thousandths of a degree at the boiling point of helium (4·2° K.) even after repeated cycling from room temperature to 1° K.

Megaspore Tetrads in a Coenopterid Fern

W. G. CHALONER has observed and described a new type of fossil spore tetrad, *Didymosporites scotti gen. et spec. nov.*, from Carboniferous strata in Ireland, Scotland and England, which may be the megaspores of the cenopterid fern *Stauropteris burntislandica* (*Annals of Botany*, N.S., 22, 197; 1958). The spores always occur as tetrads consisting of two large and two minute spores with cutinized walls, the former presumably fertile, the latter abortive. The evidence from studies of the distribution of these spores indicates that this species was much more widely distributed in time and space than has been previously known. The conformation of the coherent tetrads raises the question as to whether they were 'shed' while still within the megasporangium, as in *Lepidocarpon* and pteridosperm seeds.

Combustion Institute Awards

DR. BERNARD LEWIS, president of the Combustion Institute, Pittsburgh, Pennsylvania, is the first recipient of the Institute's Bernard Lewis Medal awarded "for brilliant research in the field of combustion, particularly on minimum ignition energy". The Alfred C. Egerton Medal of the Institute was awarded to Sir Alfred Egerton, professor emeritus of the Imperial College of Science and Technology, London, and chairman of the British Section of the Combustion Institute, "for his distinguished, continuing, and encouraging contributions to the field of combustion". The Combustion Institute Silver Medal was awarded to Prof. Seiichiro Kumagai and Hiroshi Isoda, of the University of Tokyo, in recognition of "an outstanding paper presented at the Sixth Symposium on Combustion" at Yale University in 1956. Similar awards will be made at each biennial symposium. The Eighth International Symposium on Combustion will be held in 1960 at California Institute of Technology, Pasadena.

X-Ray Analysis and Electron Microscopy of Metals

THE X-ray Analysis and Electron Microscopy Groups of the Institute of Physics will hold a joint conference on "Precipitation in Alloys" on Friday, November 28, at the Institution of Civil Engineers, Great George Street, London, S.W.1. On the previous day the Electron Microscopy Group will hold a symposium on "Electron Staining" at the Royal Society of Arts, 6–8 John Adam Street, W.C.2, while on the day following the joint conference, the X-ray Analysis Group will discuss the examination of metals by X-ray diffraction at a meeting at the Institution of Civil Engineers. An exhibition of apparatus, etc., is being arranged at the Institution of Civil Engineers. Non-members of the Institute of Physics may attend these meetings; they should complete an application form to be obtained from the Honorary Secretary of the X-ray Analysis Group, Dr. R. L. Gordon, Safety in Mines Research Establishment, Portobello Street, Sheffield 1, from whom further information may be obtained.

Announcements

MR. E. LORD of the British Cotton Industry Research Association has been awarded the Warner Medal of the Textile Institute.

DR. ROGER REVELLE, director of the Scripps Institution of Oceanography, University of California, has now been appointed to the additional post of director of the University's new Institute of Technology and Engineering at La Jolla.

PROF. R. H. S. THOMPSON, professor of chemical pathology in the University of London at Guy's Hospital Medical School, Prof. E. J. Wayne, regius professor of the practice of medicine in the University of Glasgow, and Prof. B. W. Windeyer, professor of radiology in the University of London and director of the Meyerstein Institute of Radiotherapy at the Middlesex Hospital, have been appointed members of the Medical Research Council.

THE following research studentships for post-graduate training in ecology, tenable for periods up to three years at the universities shown, have been awarded by the Nature Conservancy: *Botany*: J. M. Bradbeer (Southampton), D. Briggs (Durham), T. T. Elkington (Durham), P. J. Myerscough (Oxford), J. G. B. Newbould (Oxford), B. M. Stephens (University College of North Wales, Bangor); *Zoology*: N. G. B. Jones (Oxford), P. O'Donald (Cambridge), Miss R. M. Oldroyd (London), Miss E. A. Steel (Reading); *Geography*: J. C. Rodda (University College of Wales, Aberystwyth).

AWARDS for study in statistics by persons whose primary field is not statistics but one of the physical, biological or social sciences to which statistics can be applied are offered by the Department of Statistics of the University of Chicago. The awards range from 3,600 to 5,000 dollars on the basis of an eleven-month residence. The closing date for application for the academic year 1959–60 is February 16, 1959. Further information can be obtained from the Department of Statistics, Eckhart Hall, University of Chicago, Chicago 37, Illinois.

THE twenty-fifth Annual Conference of the National Society for Clean Air will be held in Llandudno during October 1–3. Further information can be obtained from the Society at Palace Chambers, Bridge Street, London, S.W.1.

A MEETING of the North of England Section of the Society for Analytical Chemistry will be held on October 4 at the City Laboratories, Mount Pleasant, Liverpool, 3, to discuss "Laboratory Balances".

THE Antiquarian Horological Society is holding a meeting in Birmingham on October 4. Further information can be obtained from Mr. A. K. Soper, 29 Furham Field, Hatch End, Middlesex.

THE eleventh Annual Conference on "Electrical Techniques in Medicine and Biology" will be held in Minneapolis, Minnesota, during November 19–21. It is sponsored by the Institute of Radio Engineers, the American Institute of Electrical Engineers, and the Instrument Society of America through their Joint Executive Committee on Medicine and Biology. Abstracts of two hundred and fifty words for short contributed papers offered for inclusion in the programme may be sent by October 1 to Mr. Robert Erskine, Minneapolis-Honeywell, 2753 Fourth Avenue South, Minneapolis, Minnesota, from whom further information can be obtained.

ERRATUM: In the communication entitled "Hæmoglobin *P* (the 'Galveston' Type)" by Dr. Rose G. Schneider and Dr. Mary Ellen Haggard, published in *Nature* of August 2, p. 322, the symbol μ (ionic strength) should be substituted for the symbol *M* (molar) throughout the text and in the legends of Figs. 1 and 2.

HIGH POLYMERS

AN International Conference on High Polymers was held at the University of Nottingham during July 21–24. This Conference, which was organized by a British committee under the chairmanship of Sir Harry Melville, was attended by nearly six hundred scientists, including an official delegation of twelve Russians and altogether 150 foreign specialists —from twelve nations—in the physics and chemistry of high polymers.

The Conference provided a timely opportunity for a full exchange of views between those working in the high-polymer field at universities and in industrial and government research laboratories. The main themes selected for discussion were : (*a*) reaction mechanisms and kinetics with special reference to heterogeneous polymerization, including trapped radicals. Production of graft and block co-polymers; (*b*) physical, thermodynamic and mechanical properties of high polymers. The papers, which were preprinted and circulated before the meeting, were limited to forty-four.

It is now becoming accepted practice at international polymer meetings for Prof. H. Mark of the Polytechnic Institute of Brooklyn, New York, to deliver the opening address, and on this occasion he summarized in a masterly manner recent developments in the preparation and synthesis of stereospecific polymers, and the structural importance of helical configurations in understanding the properties of these polymers.

The discovery in 1953 by Karl Ziegler that catalysts formed by interaction of a metal alkyl of groups I, II and III elements with certain transition metal compounds polymerize ethylene rapidly to a high molecular weight polyethylene at room temperature and atmospheric pressure opened up a wide and important field of the study of heterogeneous polymerization, and several papers were devoted to a study of the mechanism of the action of Ziegler catalysts. Many catalytic systems have been found to be equally effective in polymerizing ethylene and other olefins at low pressures, and one of the Russian papers dealt with the use of chromium and molybdenum oxides for this purpose. Catalysts of this type are used commercially in the United States for the synthesis of polyethylene of high molecular weight. There is much evidence that soluble complexes formed by interaction of certain titanium compounds and metal alkyls polymerize ethylene in solution at normal temperatures and pressure homogeneously, although at a rate much lower than that of typical heterogeneous catalysts. Catalysts of this kind are probably more amenable to mechanistic study, and detailed experimental kinetic results were presented on the polymerization of ethylene by homogeneous catalysts made from the interaction of aluminium triethyl and *sec*-butyl titanate, and zinc di-*n*-butyl and titanium tetrachloride in hydrocarbon solvents. Kargin and his colleagues (Moscow) reported that the mechanical properties of ethylene polymers made from catalysts formed by the interaction of organo-lithium compounds with titanium tetrachloride showed marked differences depending on the structure of the organic metallic component of the catalyst.

The most important development in the study of heterogeneous polymerization of the above type was the synthesis in 1954 by Natta and his co-workers at the Milan Polytechnic of α-olefin polymers with regular, linear, head-to-tail structures and the separation of the synthetic product into isotactic and atactic polymers. The stereospecific configuration of the former was established by X-ray methods, and a study of the nature of the catalysts enables the stereospecificity of the heterogeneous catalysts to be controlled. In this way many crystalline poly-α-olefins and di-olefins of definite stereochemical structure were synthesized for the first time. In the opening paper of the general discussion, Natta described his very extensive investigations on the kinetics of α-olefin polymerization and co-polymerization of ethylene with α-olefins. The decomposition of aliphatic diazo compounds by boron trifluoride and other boron compounds has been known for a long time to yield polymethylene of high molecular weight. From a kinetic study of this reaction Bawn and Ledwith proposed a polymerization mechanism involving boron alkyl as the true catalyst. They suggest that the polymerization is analogous to Ziegler-type polymerization of α-olefins and occurs by co-ordination with the boron, and that this results in a successive series of insertions of monomeric units into a boron–alkyl bond. An interesting example of anionic polymerization was discussed by Overberger and co-workers (Brooklyn), who postulated that the initiation process of the polymerization of methacrylonitrile in liquid ammonia is a one-electron transfer from lithium to the monomer to give an anion radical.

In 1955 it was reported in the patent literature that propylene oxide could be polymerized by ferric chloride to give products of high molecular weight which were partially crystalline. C. C. Price (1956) showed that if an optically active monomer is used the activity is retained in the crystalline polymer, and the mechanism of polymerization suggested by Price is analogous to that proposed by Natta for the heterogeneous polymerization of α-olefins. In a series of papers, C. C. Price and co-workers (University of Pennsylvania) and G. Gee and collaborators extend these investigations to other olefin oxides and show that a wide variety of catalyst systems—Lewis acid metal alkoxides, mixture of alkoxides and halides or metal halides—serve for the polymerization of propylene and other olefin oxides.

Many of the kinetic features of the polymerization of vinyl monomers in conditions in which the polymer formed is precipitated may be explained in terms of occlusion of precipitated free radicals of polymer chains. Bamford, Jenkins, Symons and Townsend reported results of the concentration of trapped polyacrylonitrile radicals as determined by electron resonance spectroscopy and the variation of this concentration with experimental conditions such as degree of swelling of the polymer, rate of photo-initiation and temperature. The fraction of radicals which become trapped was shown to be about 12 per cent in the bulk polymerization of acrylonitrile. Electron spin resonance measurements were also used by Atherton, Melville and Whiffen to follow radical concentrations in the co-polymerization of methyl methacrylate and glycol dimethacrylate.

F. S. Dainton and co-workers summarized their extensive and detailed studies on the polymerization of acrylonitrile in aqueous solution when initiated by : (*a*) Fenton's reagent, and (*b*) photochemically, using

various photosensitizers. By means of the rotating sector technique they have determined both k_p and k_t and also elucidated : (1) the mechanism of the termination reaction by salts of metals of variable valency ; (2) the effect of coagulation on the rate of polymerization. Other interesting observations on heterophase polymerization which were reported and discussed were : (*a*) the increase in rate of the high-energy initiated polymerization of isobutene by the presence of metal oxides of high surface area ; (*b*) the effect of the physical nature of the separating phase on the rate of the heterogeneous polymerization of acrylonitrile and methyl methacrylate by Palit and Guha (Calcutta) ; (*c*) the co-polymerization of water-soluble sodium methacrylate with methyl methacrylate maintained in a fine particle size with the aid of water-soluble colloids.

Block and Graft Co-polymers

The general methods of synthesis of graft co-polymers have been fairly well worked out during the past ten years, and the group of papers presented to the discussion largely elaborated and consolidated these procedures, particularly with reference to the utilization of ionizing radiation, interaction of functional groups of macromolecules with monomeric and polymeric substances, and the initiation of monomers by macro-radicals. Various syntheses of graft and block co-polymers have been described in which polymers containing peroxides, hydroperoxides and perester groups may be used as initiators for the second-step polymerization, and Prof. G. Smets and co-workers (Louvain) presented kinetic results obtained with polymethylacrylate co-polymers containing *tert*-butyl perester groups and styrene monomer. A new method of synthesis of block co-polymers proposed involved the synthesis of polymers containing peroxide end-groups and the metal salt catalysed decomposition of the peroxide group in a second monomer. Prof. A. Berlin (Moscow) described the synthesis of graft co-polymers of branched structure by the polymerization of a number of monomers in latexes of polyvinyl chloride and synthetic rubbers. The preparation and kinetics of formation of block co-polymers from a primary polymer with amine end-groups which may be used as a macromolecular transfer agent and also the formation of graft co-polymers using a primary polymer with terminal double bond which co-polymerizes with a second monomer were described by Bamford *et al.* Wichterle and Gregor (Prague) reported the preparation of polymers of N-methacryloyl caprolactam. Polymer amides prepared in this way were shown to catalyse the polymerization of caprolactam, and the resulting grafted co-polymers had a three-dimensional structure. Caprolactam reacts with styrene–methyl methacrylate co-polymers to form soluble graft co-polymers, the properties of which have been studied by Chapman and Valentine. The mechanism and properties of grafts formed by polymerizing vinyl acetate monomer in the presence of polyvinyl alcohol and an alkali and a water-soluble persulphate catalyst were discussed by Hartley, and those of polymethyl methacrylate graft to natural rubber by Kobryner and Banderet (Strasbourg).

The recent interest in the use of radiation to produce new polymer hybrids has spurred a great deal of research, and four papers presented on this subject dealt mainly with grafting of vinyl monomers to polyethylene film. In general, four methods were described for producing such co-polymers : (1) Simultaneous grafting—the film being irradiated while immersed in, or swollen by, monomer ; (2) post-irradiation grafting—irradiation of dry film *in vacuo* or in air, followed by addition of monomer. These various aspects were described in the papers by Metz *et al.* (Brookhaven), Chapiro (Paris) and Hoffman (Paris). Dr. Chapiro also described the radiation grafting of styrene and methyl methacrylate on to chemically inert polytetrafluoroethylene. Cooper and co-workers compared the graft co-polymers from natural rubber latex and methyl methacrylate using visible, ultra-violet and γ-radiation and measured the effects of changes of monomer concentration and radiation intensity on polymerization-rates and molecular weights. The production of cross-links and of sites for grafting in solid high polymers may be achieved by hydrogen abstraction using ultra-violet light (200–300 mμ). The rate is very much increased by the use of photosensitizers, and a comparative study of this effect with various aromatic carbonyl compounds was described by Oster (Brooklyn). Another method of grafting on to solid polymer was described by Natta (Milan) for poly-α-olefins. These hydrocarbons readily peroxide on heating in air, and the surface or internal hydroperoxide groupings (depending on the nature of the polymer) readily form free radical centres by thermal decomposition.

The mastication of polymers brought into a rubbery state on admixture with monomer causes polymerization of the monomer. This reaction was shown (Watson *et al.*) to have the characteristics of that expected if initiation was brought about by mechanical rupture of the polymer into free radicals. The preparation and properties of block co-polymers of polymethyl methacrylate-polystyrene were described.

Physical, Thermodynamic and Mechanical Properties

In a theoretical paper on rubber elasticity, Flory, Hoeve and Ciferri (Mellon Institute) generalized the rubber elastic equation of state for the case where the chain displays change in internal energy with change in conformation, and investigated the relationship between the temperature coefficient of the mean square end-to-end chain distance $\overline{r_0^2}$ and the elastic equation of state of the network. Gee and co-workers, developing the thesis that the effects of dynamic properties of linear polymers are closely related to intermolecular forces and chain flexibility, reported results on the measurement of the latter properties for poly*iso*butene. A torsion pendulum for measuring the internal friction of polytetrafluoroethylene was described by McCrum (Dupont). Results over the range 4·2° K. to the crystalline melting point at 600° K. indicated that hindered rotation occurs within the crystallites at temperatures as low as 220° K.

Several papers were concerned with the relationships between properties and structure. Meyerhoff and Canton (Mainz) discussed the molecular weights and molecular weight distribution of branched and unbranched polystyrene, and Magat *et al.* (Paris) extended the theory of the solubility of high polymers to solution of graft co-polymers. Natta discussed the dependence of the properties of α-olefin polymers on their steric composition and summarized extensive results on the physical and mechanical properties of random, stereo and other kinds of block co-polymers.

The technical processes used for the manufacture of polyethylene yield a polymer which shows chain

branching, the degree of branching varying considerably with the conditions of polymerization. Methods of measurement of branching have therefore been of paramount interest in characterizing ethylene polymers, and a considerable effort has been expended on these studies. In a detailed and elegant study, Willbourne (I.C.I. Plastics) summarized the preparation of model branched polymethylenes and calibration methods which have been used for developing methods of measuring the numbers and length of branches in polythene. The effect of branching on the degree of crystallinity, crystalline melting point, density, rate of oxidation and dynamic mechanical properties were illustrated and discussed. Gubler and Kovacs (Strasbourg) presented similar physical measurements on a range of commercial polythenes and made an interesting approach to an interpretation of these properties by considering the polymer as a mixture of *n*-paraffins. The orientation of crystallites which occurs on stretching polyethylene and its dependence on the degree of crystallinity were discussed by R. S. Stein (Amherst), and Eppe, Fischer and Stuart (Mainz) presented results obtained by electron diffraction and electron microscopic studies on the morphological structure of polyethylene, polyamides and other polymers.

The importance of crystallization under stress in fibre-making was emphasized in a paper by Thompson (I.C.I. Fibres), who proposed a model visco-elastic theory to explain the observed results, and de Vries (Amheim) discussed an empirical relationship between draw ratio of synthetic fibres and birefringence.

During the conference a number of technical visits were arranged to commercial laboratories and works. A reception was held on the opening evening, and the conference concluded with a dinner held in the Portland Building of the University.

C. E. H. Bawn

COMPARATIVE ENDOCRINOLOGY

During May 25–29, nearly 150 scientists from many parts of the world met at the Cold Spring Harbor Biological Laboratories, Long Island, New York, to take part in the Columbia University Symposium on Comparative Endocrinology. The conference was organized by a committee headed by Prof. A. Gorbman, of the University of Columbia, and the choice of Cold Spring Harbor provided an ideal setting for its deliberations. The scientific programme was split up into a number of sections or main topics.

The first of these was concerned with endocrine factors in ecological adaptation. Prof. W. Hoar (British Columbia) reviewed the role of the endocrine system in permitting fish to survive and adapt themselves to seasonal changes, while his research associate, Miss B. Baggerman, gave neat and conclusive proof of the involvement of salinity preferences in the migratory behaviour of fish.

In his interesting account of mammalian adaptive mechanisms, Dr. J. J. Christian (Bethesda) reported on the relation between adrenal size and population growth in rodents. A gradual numerical increase in the population of mice in cages, without overcrowding, leads to a significant enlargement of the adrenal glands accompanied by lowered reproductive activity (for example, a reduction in litter size), and thus, by inference, to an adjustment of population size. Whether the two phenomena are causally connected is not clear, and it may be that the increased size of the adrenals serves merely as an indicator of population density.

The next session dealt with the endocrine basis of reproductive adaptations in the major groups of vertebrates. Prof. L. Gallien (Paris) described the unusual reproductive habits of *Nectophrenoides orientalis*, a viviparous African frog with a gestation period of nine months. Dr. M. R. Miller (Stanford), in the course of his review of reptilian reproduction, stressed the hazards of inferring endocrine function from morphological study alone. As an illustration he referred to the virtual absence of histologically demonstrable Leydig cells, apparently not associated with androgen deficiency, in certain types of lizard.

In a characteristic contribution, Prof. F. L. Hisaw (Harvard) developed a theory covering vertebrates in general, in which he stressed the ubiquitous, but not necessarily biologically significant, occurrence of progesterone among chordates, and the gradual and independent evolution of luteal and placental function in different mammals, culminating with the largely autonomous placenta of primates.

Both the comparative morphology and physiology of the principal endocrine organs and endocrine function in developing vertebrates received considerable attention during the conference. Prof. E. Wolff (Paris) and Profs. A. Raynaud (Institut Pasteur) and L. J. Wells (Minnesota) reviewed the results of their, by now classical, experiments on somatic or endocrine growth and sexual development in mammalian embryos deprived of either the pituitary or gonads.

In the course of a comprehensive survey, Prof. A. Gorbman (Columbia) directed attention to the vagaries of thyroxine formation in lower vertebrates. Thus, certain types of fish and salamanders form thyroxine from labelled iodine administered to them only after protracted periods (lasting more than a month), while others may not do so at all. Interestingly enough, there is at least one type of frog in which the thyroid gland is completely absent but the larvæ of which none the less undergo metamorphosis.

The inadvisability of applying the results of experiments in mammals to lower animals was also emphasized by other contributors. Prof. B. Houssay (Buenos Aires) and Dr. M. R. Miller (Stanford) reviewed the effects of ablation of both the adenohypophysis and pancreas in different groups of vertebrates, and revealed the following interesting position : while the combined operation in mammals leads to the preparation universally known as the 'Houssay dog', there are important deviations from this situation in lower vertebrates. Thus, birds do not develop hyperglycæmia following removal of the pancreas. On the other hand, in the urodele amphibians (for example, *Triturus*) studied by Dr. Miller, the islet apparatus is of the purely β-cell type and pancreatectomy leads to severe hyperglycæmia. Hypophysectomy, however, does not cause the expected alleviation of the condition until after some months when all inter-renal tissue has atrophied. By contrast, the islets of lizards are of the α-type, and removal of the pancreas induces hypoglycæmia

instead of diabetes. Another point established by Dr. Miller is that lizards are highly sensitive to glucagon and insensitive to (mammalian) insulin, in this respect differing markedly from birds.

Similar species differences were also indicated by Dr. S. I. Segal (New York) in the course of a stimulating account which ranged far and wide over the comparative aspects of gonadotrophic action and gonadal function in mammals and birds.

It was no more than a logical step to proceed from this point to a consideration of biochemical variations of hormones among vertebrates, which formed the subject of the next session. Dr. I. I. Geschwind (Berkeley) gave a detailed review of variations in such protein and polypeptide hormones as oxytocin and vasopressin, or the melanophore-stimulating principle of the anterior pituitary, as well as of the methods available for their study. Investigations of this kind may be expected to provide an explanation of the fact that a given hormone preparation such as somatotrophin from one mammalian group may be ineffective in another, or why one hormone (for example, prolactin) may induce wholly different responses in different species.

Neuro-endocrine reflex and co-ordinating mechanisms were the topics of another session. Prof. G. W. Harris (London) discussed the hypothalamic control of thyroid-stimulating hormone secretion, and there were separate accounts of the hormonal requirements for ovulation in amphibia (Dr. C. Y. Chang and Prof. E. Witschi, Iowa), birds (Prof. A. V. Nalbandov, Illinois) and mammals (Dr. J. W. Everett, Duke). The last-named reported in detail on the ability of pituitary autografts to the kidney to become vascularized and resume their functional activity when transplanted near the median eminence of rats. These animals, he indicated, not only resumed œstrous cycles of roughly normal duration but also ovulated, and some, when mated with males, became pregnant. By contrast, transplantation of these grafts to the temporal lobe did not restore cycles.

In a similar context, Dr. K. G. Wingstrand (Copenhagen) referred to experiments in which the adenohypophysis of toads was grafted on the site from which the median eminence had been previously extirpated, and after some weeks resumed its function. This seemed to prove that the anterior lobe must be activated by substances originating in some hypothalamic area other than the median eminence, and that the latter is only a release organ.

Neurosecretion and its role in neuro-endocrine integration were discussed by Profs. E. and B. Scharrer (New York). In their view, many vital processes are not controlled exclusively by either nervous or hormonal mechanisms, but by both. Neither works as a closed system; the neurosecretory cells provide a hormonal link between nervous centres and endocrine glands, whereby their activities are intimately interrelated.

A novel and attractive feature of the conference was a further session devoted to vertebrate organs of an unusual nature or incompletely established endocrine status, such as the ultimobranchial body, Stannius corpuscle, etc. Dr. F. Baker (Columbia), in reviewing the renal thyroid of fishes, described a condition of hyperplasia and aberrant distribution of thyroid follicles of fish kept in water with a low-iodine content. Thyroid masses, developed as a result of such compensatory hypertrophy, may be found in such ectopic sites as the eye, heart and brain, but this can be prevented by transferring the animals to a high-iodine environment.

During the same session, Prof. E. J. W. Barrington (Nottingham) gave a most stimulating and beautifully illustrated account of his bio- and histo-chemical studies on iodine binding in the endostyle of protochordates. He not only showed convincingly that this, indeed, occurs (for example, in amphioxus and tunicates), but also pointed out some interesting theoretical implications of his findings. For example, since iodine binding in amphioxus is associated with the production of an alimentary type of mucus, elaboration of thyroglobulin (which followed phylogenetically later) may have been the outcome of cytochemical evolution, perhaps as an ecological adaptation by chordates to life in fresh water. With regard to the origin of the iodination process, it is of interest that iodine binding also occurs in the surface layer of the tunic of tunicates, possibly as a random binding to scleroprotein. Since, however, the tunic surface is being continually worn away, it is conceivable that the iodination products might, by ingestion, have become incorporated by the animals, at some stage of evolution, into their biochemical processes. This, in turn, may have established the need for a more concentrated and efficient iodination process, and thus have initiated the iodinating function of the endostyle, itself the forerunner of the vertebrate thyroid gland. Although speculative at present, these conclusions may be given a firmer basis by future research.

The increasing use now being made of electron microscopy in the investigation of ultra-structural detail was very evident during the conference. A considerable proportion of contributors had included electron microphotographs, some of the best being those of Dr. J. D. Green (Los Angeles) and Sir Francis Knowles (Marlborough College). The standard of illustrations, in general, was remarkably high. There were many admirable colour slides—few perhaps finer than those shown by Prof. Barrington and Drs. Miller and Knowles. The superb colour film of the rat's ovary during the process of ovulation prepared by Prof. R. J. Blandau (Washington) must also be mentioned in this connexion.

The official proceedings of the entire conference will be issued as a separate volume, publication of which is confidently predicted before the end of the year.

P. ECKSTEIN

GLASGOW UNIVERSITY EXPEDITION TO NORTH RONA AND SULA SGEIR

AN expedition to the remote, uninhabited Scottish islands of North Rona and Sula Sgeir (45 miles north-west of Cape Wrath) took place during June 23–July 23. Arrangements for the expedition were made largely by four members of the University of Glasgow, D. E. Baird, I. O. Macdonald, R. S. Wyburn and S. B. Donald. The other six members of the expedition were Miss Helen C. Nisbet and R. A. Gailey

(University of Glasgow); Mr. and Mrs. M. Lauder-Smith; T. B. Bagenal and H. T. Powell (Marine Station, Millport). The present account refers principally to work carried out on North Rona; one day only was spent on Sula Sgeir.

North Rona is composed of Lewisian gneiss, granulitic in texture and showing strong differentiation into acidic and basic fractions. A notable feature is the occurrence of sheet-like intrusions of quartz–felspar pegmatite with a coarse graphic structure, the outcrops of which are largely controlled by folding along W.N.W.-trending axes. Ample evidence for glaciation exists, but no indication of the direction of ice movement could be found. A thin cover of boulder clay, containing erratics of mainland rocks, exists over most of the island. Remnants of wave-cut platforms were noted, and the approximate heights of the principal morphological features were determined.

The expedition made a detailed ground survey of the remains of Saint Ronan's cell and chapel, the church enclosure, and the adjacent village which has been deserted since 1844. The cell is rectangular with sloping walls and a roof of flat slabs, and may date from the eighth century; the adjoining chapel is later in date and has been used as the village church. The original foundation was probably a hermitage. The village is situated towards the north-west corner of an infield of some 24 acres, cultivated in large lazybeds. There are three groups of ruins in the village, each consisting of a rectangular dwelling surrounded by a number of small circular or oval cells.

A survey of the terrestrial flora was carried out and a collection made of the flowering plants, bryophytes and lichens. Only 44 species of flowering plants were found on North Rona, and Sula Sgeir has only seven. Soils were examined *in situ*, and samples brought back for further study. A comprehensive collection of terrestrial invertebrates was made, and ecto- and endo-parasites were collected from some of the 140 sheep on Rona. Selected blood samples and blood-sucking insects were taken for the Ruchill Virus Research Laboratory.

A survey of the marine flora and fauna was made on both islands and, considering the isolation of the islands and the very severe exposure to swell and wave action, a surprisingly large number of algae and invertebrates was found. One of the rarest and most interesting algae present was *Fucus distichus* subsp. *edentatus*. North Rona is the largest known breeding ground of the Atlantic grey seal, and it is of interest to record that one seal calved successfully as early as July 6; the earliest calves recorded hitherto for Britain have been born in mid-August.

A census was made of the birds breeding on Rona and notes kept of the migrants passing through. Although counts of those species nesting in the sea caves were not always possible, many of the figures obtained show marked changes from the counts made before the War. Leach's petrel was of special interest, for in Britain it breeds on only very few Scottish islands, including North Rona and Sula Sgeir; its distribution and numbers on Rona were estimated, and some 500 were ringed. Of the migrants, the most spectacular was a red-headed bunting that remained on the island for a few days.

Two films and many photographs were taken of features of interest on the islands. The results of the various investigations are now being worked up and will be published elsewhere.

The expedition is grateful to the University of Glasgow, the Carnegie Trust for the Universities of Scotland, Mr. Charles Hepburn, and other individuals, institutions and firms, for financial or other practical support for the venture; to Commander R. C. Dumas and the crew of M.F.V. 289 for invaluable assistance in getting to and from North Rona; and to Dr. R. R. Morrison of Bridge-of-Weir for transporting six members of the expedition from Rona to Sula Sgeir aboard his motor-yacht *Mary Rose of Morar*.

T. B. Bagenal
H. T. Powell

THE INSTITUTE OF PHYSICS

THE thirty-eighth annual report of the Board of the Institute of Physics, covering the work of the Institute during 1957, was presented to the annual general meeting of the Institute on July 1. Particular attention is directed in the report to the steady growth in the membership of the Institute during the past ten years, from 3,455 in 1948 to 5,881 in 1957. The number of applications for election or transfer to the various grades of membership received during the year under review for submission to the membership and examinations committee was 795 compared with 752 in the previous year. Representatives of the committee visited nine technical colleges which had applied for recognition by the Institute. Five colleges requested the recognition of diplomas awarded on full-time or sandwich courses as satisfying the Institute's academic requirements for membership, and two diplomas, the awards of Sunderland Technical College and the Welsh College of Advanced Technology, Cardiff, were accepted. The Board accepted the recommendation of the membership and examinations committee that students following courses in recognized institutions leading to qualifications recognized by the Institute should be eligible for admission to the student grade of membership irrespective of compliance with the Institute's regulations concerning general education, but students who do not comply will still be required to do so before being eligible for the graduateship grade of membership.

In the written papers for the examination for the graduateship grade twenty-one of the sixty-eight candidates satisfied the examiners. There were 542 candidates for the Ordinary National Certificate in applied physics and 182 for the Higher Certificate, compared with 424 and 152, respectively, in 1956.

The circulation, both to members and non-members, of the Institute's two journals, the *Journal of Scientific Instruments* and the *British Journal of Applied Physics*, increased during the year. Supplement No. 6 of the *British Journal of Applied Physics*, containing a selection of the papers presented to the Institute's Non-Destructive Testing Group, including most of those read at the Group's conference held in Bristol in July 1956, was published in May. In the "Physics in Industry" series, a new volume "Pressure Measurement in Vacuum Systems", by J. H. Leck, was published during the year, together with a revised edition of Dr. J. Topping's "Errors of Observation and their Treatment" in the "Monographs for

Students" series. "A Further Handbook of Industrial Radiology" written by fifteen members of the Non-Destructive Testing Group and edited by W. J. Wiltshire, and a fourth selection of "Laboratory and Workshop Notes" reprinted from the 1953–55 *Journal of Scientific Instruments*, compiled and edited by Dr. Ruth Lang, were published by Edward Arnold (Publishers), Ltd., for the Institute.

The third convention of the Institute, held in Oxford during July 10–13, was attended by about 250 members, ladies and guests, a rather smaller attendance than expected. Lectures were delivered by Prof. N. F. Mott on dislocations and the physics of materials and by Prof. A. C. B. Lovell on recent advances in radio-astronomy. The address given by Sir Cyril Hinshelwood on July 11 in the Sheldonian Theatre on "Physics among the Sciences" was published in the September issue of the *Bulletin* (8, 300; 1957) and the lecture by Mr. Alan Bullock on July 12 on "Science and the Humanities" in the December issue (8, 380; 1957).

The Institute has nine branches in Great Britain, a Malayan branch under the chairmanship of Mr. Hon Yung Sen and an Australian branch with divisions in different states under the presidency of Dr. F. W. G. White. The New South Wales division formed a solid-state physics group, the first specialist group to be formed in the Australian branch. In addition to the branches in Great Britain, there are seven specialist subject groups—applied spectroscopy, education, electronics, electron microscopy, non-destructive testing, stress analysis and X-ray analysis—which arrange their own programmes of scientific meetings and conferences.

The Board through its permanent officers has continued to give advice in answer to individual inquiries concerning professional matters, and inquirers requesting assistance in filling vacancies for physicists were advised to advertise in the *Bulletin*, which has now become a recognized medium for this purpose. In reply to the Scientific Manpower Committee of the Government's Advisory Council on Scientific Policy the Board stated that the members of the Board were generally agreed that if the number of graduates in physics were doubled within five to ten years the demand could still exceed the supply, and that the Board was perturbed by the number of first-class physicists who were leaving the country for permanent employment overseas. The results of the fourth survey of salaries and emoluments paid to fellow, associate and graduate members of the Institute were published in the January issue of the *Bulletin* (8, 19; 1957) and reprints were widely distributed. Discussions with the Physical Society for closer collaboration between the Institute and the Society have progressed favourably, and an announcement of interest will shortly be made.

To mark the completion of twenty-five years as secretary and editor, and in recognition of his services to the Institute, a presentation was made by the Board to Dr. H. R. Lang.

At the general meeting of the Institute, the following were elected to take office on October 1: *president*, Sir George Thomson; *vice-president*, Dr. R. W. Sellars; *hon. treasurer*, Dr. J. Taylor; *hon. secretary*, Prof. F. A. Vick. Sir William Lawrence Bragg was elected to Honorary Fellowship.

S. WEINTROUB

COUNCIL FOR THE PRESERVATION OF RURAL ENGLAND

THE thirty-second annual report* of the Council for the Preservation of Rural England, covering the period May 1957 to April 1958, again stresses the impact which the nuclear power programme is likely to have on the appearance of the countryside. Probably less than a tenth of the population are directly concerned with the land, and it is the urban majority which dictates, in the main, the shape of future developments. The Council welcomes the formation, on Mr. Duncan Sandys's initiative, of the Civic Trust for the encouragement of good architecture and civic planning and the stimulation of interest of urban and suburban dwellers in the visual aspect of town and country and the avoidance of unsightly development. The work of the Council itself continued along the same general lines, but the volume and range of work undertaken continued to increase.

The report provides a very useful summary of the bearing on the preservation of the countryside of recent legislation, such as the Electricity Act, 1957, the Winfrith Heath Act, 1957, the Local Government Bill, the Opencast Coal Bill, the Tribunals and Inquiries Bill and the Water Bill. Considerable discussion and correspondence has taken place between the Council and the Ministry of Housing and Local Government in regard to the recommendations of the Franks report relating to inspectors' reports, and the Ministry has agreed to supply the Council or its branches with copies of such reports on request. The use of land by Service Departments continued to demand much attention, and concern is expressed at the tendency of the Electricity Authorities to adopt the line of least resistance. The protracted negotiations regarding the routing of the electricity supply to the television station on Sandale Fell (Cumberland) are described in some detail as illustrating the meticulous care taken by the Minister of Power to give effect to his undertaking to try to reconcile the claims of landscape beauty with the urgent need to provide electricity in rural areas as economically as possible.

Numerous other instances are cited in the report in which the Council has had to raise objections, and mineral undertakings and roads and footpaths have also been the source of much anxiety, particularly the use of the Kendal to Keswick road over Dunmail Raise by heavy industrial traffic. The Council urges strongly that heavy industrial traffic should be discouraged from using this road by suitable improvements to the west coast road to make this an attractive alternative, and also by developing the alternative route through the Lune valley. This matter is regarded as particularly urgent, and the problem is being thoroughly investigated by traffic experts of the Automobile Association and the Royal Automobile Club.

* Council for the Preservation of Rural England. Thirty-second Annual Report, 1957–1958. Pp. 64+4 plates. (London: Council for the Preservation of Rural England, 1958.)

COLLECTED PAPERS OF THE ROWETT RESEARCH INSTITUTE

THE work done at a large research centre such as the Rowett Institute is necessarily published in a wide variety of journals. It is therefore very helpful when, at regular intervals, a collection of its recent publications becomes available. Volume 14 of the Rowett Institute papers has just been issued*. It contains reprints of more than 60 papers and cites another 30 by title only. Practically all the 94 publications appeared in 1956 and 1957. Several of them will be of interest mainly to the practical man since they deal with subjects of such considerable practical importance as winter grazing in north-east Scotland, methods of extending the grazing season, the rearing of calves and the management of the breeding ewe. A large proportion of the papers, however, are devoted to the valuable basic research work done by the Institute on subjects such as the biochemistry, physiology and microbiology of the rumen, water metabolism in the sheep and energy expenditure in livestock. Some of the other fundamental work described in the collected papers is concerned with the metabolism of short-chain fatty acids and with enzyme studies, particularly work on the glucuronidases.

An important feature of the volume is the fact that it contains a summary extending to 54 pages which is also issued separately from the bound volume and which can be obtained by applying to the Rowett Research Institute. This separate summary begins by giving an account of the history and aims of the Institute, and contains not only a list of all the papers that are collected together in the bound volume with their titles and references, but also a concise account of the work they describe written by the director, Dr. D. P. Cuthbertson. The summary contains also two review articles which are in fact progress reports on two sections of the Institute's work. One deals with glycosidases and their inhibition by aldonolactones and has been written by Dr. G. A. Levvy. The other is a description by Dr. J. Duckworth of the work his Department has been doing on the skeleton in lactation and growth. Both the bound volume and the separate summary can be highly recommended to all who are interested in the recent advances that are now being made, not only in many practical aspects of animal nutrition, but also in the fundamental principles on which the subject is based.

* Rowett Research Institute. Collected Papers, Vol. 14. (94 papers.) Summary and Subject Reviews. Pp. ii+54. (Bucksburn, Aberdeenshire: Rowett Research Institute, 1958.)

RIBONUCLEOPROTEIN PARTICLES FROM *ESCHERICHIA COLI*

By Dr. A. TISSIÈRES and Dr. J. D. WATSON
Biological Laboratories, Harvard University, Cambridge, Massachusetts

THE amount of ribonucleic acid in exponentially growing *Escherichia coli* cells with a generation time of about 30 min. is of the order of 25 per cent of the dry weight of the organism. This value varies inversely with the time of generation and seems directly related to the synthetic ability of the cell[1–3]. About 90 per cent of this ribonucleic acid is bound to the ribonucleoprotein particles, which thus form a major constituent of the bacterial cytoplasm. These particles were first described on electron micrographs of *E. coli* lysed by phage[4], and later it was discovered that they contain the bulk of the ribonucleic acid of the cells[5]. They have been found in extracts of all bacterial species so far examined[5,6]. They resemble closely by their size, chemical composition, and several other properties the ribonucleoprotein particles which have been studied in detail in animal and plant tissues[7–10] and in yeast[11], and which are generally believed to be the site of protein synthesis[12,13].

In some animal tissues, such as the pancreas, most of the ribonucleoprotein particles are attached to the reticulum and they can only be isolated after dissolving the membranous material with deoxycholate[8]. In other tissues, however, most of the particles appear to be free in the cytoplasm[14]. The significance of this difference is not known. In bacterial extracts the particles are free. This suggests that they are either free in the cytoplasm or else attached to a very loose structure which collapses when the cells break open.

The early experiments of Schachman, Pardee and Stanier[5] with bacterial extracts indicated the presence of two main fractions with sedimentation constants of 29*S* and 40*S*, which suggested that the bacterial particles might be smaller than those from other sources. Recently, however, several investigators (ref. 15 and personal communication from Spiegelman, S.) have observed the presence of larger particle fractions, characterized by 60*S* and 80*S* when the bacterial extract contained a high concentration of magnesium.

The experiments to be reported here confirm the stabilizing role of magnesium, and indicate that under suitable conditions four types of ribonucleoprotein particles may be observed (32*S*, 51*S*, 70*S* and 100*S*). The 70*S* particle will be shown to break down into the 32*S* and the 51*S* components. Both these smaller units can be obtained in stable conditions, and their properties, as well as those of the 70*S* and 100*S* particles, will be reported and discussed. The sedimentation constants given here were corrected to 20° C. and zero concentration. They are 10–20 per cent higher than those of other workers. This may possibly be explained by the fact that they were measured on isolated particle preparations.

Preparation of Extracts and of Ribonucleoprotein Particles

E. coli strain *B* was grown in broth, at 37° C., under forced aeration. The cells were harvested in

the logarithmic phase of growth and washed twice in the refrigerated centrifuge with the buffer used to make the extract. In most cases, the cell-free extract was made by grinding the cells at 4° C. with three parts of alumina powder and extracting with three to six volumes of buffer. Deoxyribonuclease (1γ/ml.) was always added to depolymerize the highly viscous bacterial deoxyribonucleic acid. The procedure for breaking the cells was, however, not critical: extracts made by using sonic vibrations, or by lysing the protoplasts formed following the addition of penicillin to the culture medium[16], gave identical sedimentation patterns.

The crude extract, which is the supernatant after centrifugation at 6,000*g* for 15 min., contains the bulk of the ribonucleic acid of the cells, and only small amounts of it are found in the large cell debris present in the sediment and formed of pieces of cell membranes and cell walls. The supernatant, after centrifuging the crude extract at 100,000*g* for 120 min., contains some low-molecular weight ribonucleic acid in amounts of about 10–15 per cent of the total ribonucleic acid of the crude extract, whereas the major part of this substance is found in the pellet in the form of ribonucleoprotein particles. This pellet is now re-suspended in buffer, and the particles are freed of soluble proteins by successive washings in the centrifuge at 100,000*g*.

Sedimentation Diagrams

When the crude extract is made in 0·01 *M* magnesium acetate and 0·001 *M* *tris* (hydroxymethyl) aminomethane *p*H 7·4, and the particles from it are resuspended in 0·005 *M* magnesium and dilute *tris*, the schlieren diagram shows a major peak with a sedimentation constant of 100*S*. In addition there is a small amount of a 70*S* peak.

If the particles are resuspended in dilute *tris* containing 0·001 *M* magnesium, there is a major 70*S* peak and small amounts of 32*S*, 51*S* and 100*S* peaks. At a still lower effective magnesium concentration (this is the case with 0·001 *M* magnesium together with 0·01 *M* phosphate buffer, *p*H 7·0) the bulk of the ribonucleoprotein particles is present as 32*S* and 51*S* components. The 70*S* and the 100*S* peaks are not visible. At these magnesium concentrations the transitions are freely reversible, and they can be obtained by dialysis against lower or higher magnesium concentrations. However, at less than 10^{-4} *M* or more than 2×10^{2} *M* magnesium, irreversible damage occurs and the particles break down to smaller pieces.

The 32*S* and the 51*S* peaks always appear simultaneously and, as judged by the area under the schlieren curve, the mass of the 51*S* fraction is double that of the 32*S* one. This, together with the fact that the molecular weight of the 32*S* particle is probably half that of the 51*S* one (see below), would indicate that each 70*S* particle is formed of one 51*S* and one 32*S* component. The relation between 70*S* and 100*S* particles is most likely a monomer–dimer transition. Electron micrographs of 100*S* preparations show large numbers of spherical particles in groups of two, whereas 70*S* preparations reveal a majority of isolated spherical bodies (Dr. C. Hall, personal communication).

Isolation of the 51S Component

The crude extract, prepared in 0·01 *M* phosphate buffer *p*H 7·0 and 0·001 *M* magnesium, is centrifuged at 100,000*g* for 120 min. The supernatant containing the soluble proteins is poured off, and the pellet is resuspended in the same buffer. This is now centrifuged at 100,000*g* for 90 min., the supernatant is poured off, and the pellet is resuspended as above. This step is repeated twice. Finally, the preparation is brought to *p*H 5·0 by addition of 0·1 *N* acetic acid and it is centrifuged for 30 min. at 20,000*g*. The supernatant is collected and neutralized with dilute ammonia. The 51*S* preparation thus obtained is pure according to the following criteria: (1) it shows only one peak in the centrifuge; (2) it moves as a single peak in the electrophoresis apparatus between *p*H 4·7 and 7·6; (3) it shows only one kind of particle on electron micrographs (Dr. C. Hall, personal communication).

Isolation of 70S–100S Components

The crude extract was made with 0·01 *M* magnesium ion and 0·001 *M* *tris* buffer. Under these conditions the 32*S* and the 51*S* peaks are not visible on the sedimentation diagrams, and the 100*S* peak is higher than the 70*S*. This extract is then centrifuged at 78,000*g* for 120 min. The supernatant is poured off and the pellet, which is much larger than that obtained when the extract is made in phosphate, is resuspended in *tris* buffer and 0·005 *M* magnesium acetate. The mixture is centrifuged again at 78,000*g* for 120 min. This last step is repeated twice, and finally the pellet is resuspended in *tris* containing 0·002 *M* magnesium and centrifuged at 15,000*g* for 30 min. The 32*S* and the 51*S* peaks are not detectable in the supernatant. There is mostly a 70*S* peak with, in addition, 10–20 per cent of a 100*S* peak.

Properties of the Particles

Chemical composition. Ribonucleic acid and protein estimations were carried out on the purified preparations by the orcinol[17] and biuret[18] methods respectively, together with determinations of dry weights. Both the 51*S* and the 70*S*–100*S* particles were found to contain 60–65 per cent of ribonucleic acid and 40–35 per cent of protein. In each experiment the dry weight amounted to the sum of the weights of ribonucleic acid and protein within 1 or 2 per cent. Thus the particles are formed mostly, if not exclusively, of ribonucleic acid and protein.

The density of the 32*S* particles is the same as that of the 51*S*: both components sedimented at the same relative rate in a sucrose solution with a density of 1·25 as in 0·01 *M* phosphate. Thus it is likely that the four components are formed of ribonucleic acid and protein in the same proportion.

Are enzymes present in the particles? The results available are quite insufficient to decide this point. In bacterial preparations some respiratory enzymes have been found in the small-particle fraction rich in ribonucleic acid[19,20]. However, it has recently been reported that purified ribonucleoprotein particles are free from these enzymes[21]. We made the same observation: the reduced diphosphopyridine nucleotide oxidase activity disappears during the purification of the particles and the pure preparations are free from this enzyme system. The results show that the particles supporting the oxidizing enzymes are not homogeneous in size. This indicates that they are probably derived from a larger structure which breaks down to small pieces when the bacterium bursts open. It is likely that this structure is the cell membrane.

Stability in various conditions. The 51*S* and 70*S*–100*S* components are remarkably stable provided the required amount of magnesium is present. They are stable for more than two months in the cold in the presence of streptomycin or in the frozen state. The 51*S* preparation is stable for 24 hr. at *p*H 5·0 or 9·0, whereas the 70*S*–100*S* components break down under these conditions. If the magnesium concentration is lowered, or if ethylenediamine tetraacetate is added, the 51*S* particles initially break down into two components of 42*S* and 18*S*. The 32*S* component does not appear during this process. All four kinds of particles are affected irreversibly by salt concentration of the order of 0·1 *M*.

1–5 μgm./ml. of ribonuclease, at *p*H 7·6, had no effect on most 51*S* preparations, as judged by the appearance of the peak in the analytical centrifuge. In a few cases, however, the 51*S* peak disappeared after this treatment. This indicates that the particles can exist in somewhat different states. Generally, both the 32*S* and the 70*S*–100*S* particles were more sensitive to the action of the enzyme, but the rate at which they were affected was always considerably slower than would be the case with pure ribonucleic acid.

Sedimentation and diffusion constants. The sedimentation constants of all four components were measured both with the schlieren and the ultra-violet optics, with concentrations varying from 2 mgm./ml. down to 40 μgm./ml. In most cases, the values fell within about 2 per cent of those given above. Under these conditions the dependence on concentration was no more than 5 per cent. The salt concentration had no appreciable effect between 0·01 and 0·05 *M* phosphate and 0·001 and 0·05 *M tris* buffer.

The diffusion constant, $D_{20} \times 10^7$, of the 51*S* component was found to be 1·98. It was independent of the concentration between 0·07 and 0·20 per cent. The partial specific volume, measured with a 25-ml. pycnometer, was 0·66. Thus the molecular weight of the 51*S* particles is $1{\cdot}85 \times 10^6 \pm 10$ per cent.

The molecular weight of the 32*S* particles is about $9{\cdot}5 \times 10^5$, which is half that of the 51*S* component. This value is based on the sedimentation constant together with a rough estimation of the diffusion constant by following the spreading of the boundary during centrifugation. Thus, the molecular weight of the 70*S* component, which appears to be formed of one 32*S* and one 51*S* particle, is approximately $2{\cdot}8 \times 10^6$.

Similarity of the Bacterial, Animal and Plant Ribonucleoprotein Particles

The reversible breakdown of the 70*S* particles to the 32*S* and 51*S* components parallels the behaviour of other ribonucleoprotein particles. In both yeast[22] and pea seedlings[10], 80*S* particles break down reversibly to 60*S* and 40*S* units. It is likely that these experiments express a general phenomenon: the presence of a principal ribonucleoprotein particle of 70–80*S* and its construction from two unequal units, one twice the mass of the other.

The sedimentation constant of the 70*S* component is lower than the values given for liver (77·5*S*)[9], pea seedling (74*S*)[10], and yeast particles (80*S*)[11]. The chemical composition reported in the literature varies. For example, about 40 per cent ribonucleic acid is found in pea seedlings[10], liver[9] and yeast particles[11], whereas in the rabbit appendix they contain 65 per cent ribonucleic acid[23].

If the molecular weight of the 70*S* component is $2{\cdot}8 \times 10^6$, the ribonucleic acid content in molecular weight units is about $1{\cdot}7 \times 10^6$. This figure is very similar to that calculated for yeast and plant particles on the basis of a molecular weight of 4×10^6 and a ribonucleic acid content of 40 per cent. Thus it may be that the amount of ribonucleic acid in the particles is fairly constant, whereas the amount of protein can vary with the origin of the cells.

Summarizing, we conclude that:

(1) The ribonucleoprotein particles from *E. coli* are very similar to those of yeast, plant and animal cells. (2) For stability they need the presence of magnesium ions, and the requirement of the bacterial particles seems greater than that of particles from other sources. (3) On varying the magnesium concentration four different kinds of particles are observed with sedimentation constants of 32*S*, 51*S*, 70*S* and 100*S*. (4) The 70*S* particle is formed of one 32*S* and one 51*S* component. (5) The 100*S* particle is an aggregate of two 70*S* components. (6) The four kinds of particles appear to have the same chemical composition: they contain 60–65 per cent ribonucleic acid and 40–35 per cent protein. (7) The molecular weight of the 51*S* component is $1{\cdot}85 \times 10^6 \pm 10$ per cent; those of the 32*S* and 70*S* units are approximately $0{\cdot}95 \times 10^6$ and $2{\cdot}8 \times 10^6$ respectively. (8) The particles are relatively stable to ribonuclease.

We wish to thank Dr. J. T. Edsall for his encouragement and for the use of the analytical centrifuge, Miss B. Hollingworth for diffusion and electrophoresis measurements, and Dr. D. M. Skinner for her assistance in some of these experiments. We are also indebted to Dr. S. Spiegleman for supplying us ultracentrifuge data on his particle fractions. The work has been supported by a grant from the U.S. National Science Foundation.

[1] Malmgren, B., and Heden, C., *Acta Path. Microbiol. Scand.*, **24**, 446 (1947).
[2] Caldwell, P. C., Mackor, E. L., and Hinshelwood, C., *J. Chem. Soc.*, **4**, 3151 (1950).
[3] Brachet, J., "The Nucleic Acids", **2**, 476 (Academic Press, Inc. New York, 1954).
[4] Luria, S. E., Delbrück, M., and Anderson, T. F., *J. Bact.*, **46**, 57 (1943).
[5] Schachman, H. K., Pardee, A. B., and Stanier, R. Y., *Arch. Biochem. Biophys.*, **38**, 245 (1952).
[6] Bock, R. M., and Gilchriest, W. C., *Fed. Proc.*, **17**, 193 (1958).
[7] Palade, G., and Siekevitz, P., *J. Biophys. Biochem. Cytol.*, **2**, 171 (1956).
[8] Palade, G., and Siekevitz, P., *J. Biophys. Biochem. Cytol.*, **2**, 671 (1956).
[9] Petermann, M. L., and Hamilton, M. G., *J. Biol. Chem.*, **224**, 725 (1957).
[10] Tso, P. O. P., Bonner, J., and Vinograd, J., *J. Biophys. Biochem. Cytol.*, **2**, 451 (1956).
[11] Chao, F. C., and Schachman, H. K., *Arch. Biochem. Biophys.*, **61**, 220 (1956).
[12] Littlefield, J. W., Keller, E. B., Gross, J., and Zamecnick, P. C., *J. Biol. Chem.*, **217**, 111 (1955).
[13] Littlefield, J. W., and Keller, E. B., *Fed. Proc.*, **15**, 302 (1956).
[14] Palade, G., *J. Biophys. Biochem. Cytol.*, **1**, 59 (1955).
[15] Bolton, E. T., Hoyer, B. H., and Ritter, D. B., Abstracts, Biophys. Soc. meeting (1958).
[16] Lederberg, J., *Proc. U.S. Nat. Acad. Sci.*, **42**, 574 (1956).
[17] Dische, Z., *J. Biol. Chem.*, **204**, 983 (1953).
[18] Gornall, A. G., Bardawill, C. T., and David, M. M., *J. Biol. Chem.*, **177**, 751 (1949).
[19] Alexander, M., *Bact. Rev.*, **20**, 67 (1956).
[20] Tissières, A., Hovenkamp, H. G., and Slater, E. C., *Biochim. Biophys. Acta*, **25**, 336 (1957).
[21] Cota-Robles, E. H., Marr, A. G., and Wilson, E. H., *J. Bact.*, **75**, 243 (1958).
[22] Chao, F. H., *Arch. Biochem. Biophys.*, **70**, 426 (1957).
[23] Takata, K., and Osawa, S., *Biochim. Biophys. Acta*, **24**, 207 (1957).

FOLLICLE ARRANGEMENT IN SKIN FROM WILD SHEEP, PRIMITIVE DOMESTIC SHEEP AND IN PARCHMENT

By Dr. M. L. RYDER

Biology Department, Wool Industries Research Association, Torridon, Headingley, Leeds 6

RYDER[1] showed that in the Mouflon the secondary follicles were between the primaries instead of lying more towards one side of them, as they commonly do in many animals and in highly evolved domestic sheep. Skin has now been examined histologically from each of the main types of wild sheep, namely, Bighorn (*Ovis canadensis*), two varieties plus one unknown; Argali (*O. ammon*), three plus one unknown; Urial (*O. orientalis*), two plus two unknown; and Mouflon (*O. musimon*), three specimens.

In addition, skin has been examined from Mouflon × Soay, Soay, Shetland, a hairy West African sheep, an English and a Nigerian domestic goat, and a Barbary lamb (*Ammotragus lervia lervia*). Although much of the skin was from dried museum specimens, good preparations have been obtained, Sandison's[2] softening fluid being helpful, as it was with parchment.

All the wild sheep (Fig. 1) had secondaries between the primaries, and the primaries were in straight lines, in trios or often in groups of five. The follicles were disposed at much more acute angles than in domestic sheep, and there was an extremely large difference in diameter between the primary and secondary fibres. The primaries were coarser than the coarsest fibres of domestic sheep, while the secondaries were as fine as the finest Merino.

Fig. 1. Horizontal section of Urial sheep skin showing smaller secondary follicles between the primaries. (× 140)

The 'hairy primitive' domestic sheep have a large difference in diameter between the primaries and secondaries; the Black Headed Persian depicted by Duerden[3] had a strong tendency for the secondaries to lie between the primaries, and the same arrangement was found in a sheep skin from the Cameroons, believed to be from the West African Dwarf. In these, therefore, the secondaries seem to be beginning to move from between the primaries.

The Soay, a 'woolly primitive' domestic type, had clear primary trios, appreciable difference in diameter between the primaries and secondaries, and the secondaries were grouped in two wedges, only the points of which were between the primaries (Fig. 2*e*). Such an arrangement is often found in the Scots Blackface, whose latticed medullæ make it somewhat similar to the hairy group. The arrangement in the Mouflon × Soay (Fig. 2*c*) was similar to that of the wild sheep, having apparently reverted to the more primitive condition. But the primary fibres were not as coarse and did not have latticed medullæ. The Shetland had an arrangement similar to that of the Soay, but the fibres were on the whole finer, and there was less difference in diameter between the primaries and secondaries (Fig. 2*d*).

The evolutionary trend seems, therefore, to have been a movement of secondaries from between the primaries accompanied by a tendency for the primaries to move closer together and to lie on an arc. As more secondaries have developed in finer-woolled types, these have spread away from the primaries. The goat's arrangement was similar to that of the hairy domestic sheep (Fig. 2*a*) and their coats are not dissimilar. The lattices of the goat medullæ were, however, smaller and more regular than in sheep. It is interesting that the Barbary (Fig. 2*b*) was like the goat, because it suggests that the genus *Ammotragus*, considered to be intermediate between *Ovis* and *Capra*, shows, so far as follicle arrangement is concerned, greater affinity to goats than sheep.

Previous work on the ancestry of domestic sheep has neglected the skin. However, these results obtained with dried skin suggested that the examination of ancient material might provide a useful new approach. Good preparations were obtained from the skin of an ancient Egyptian mummified cat, so it would seem worth while examining any skin that is available from mummified sheep. Few follicles were found in leather, but excellent preparations were obtained from parchment. Fewer follicles were found in modern than in ancient parchment, possibly owing to the greater efficiency of the modern de-hairing process. Samples of parchment are difficult to obtain, and the only ones examined so far are: a specimen from some Aramaic documents of the fifth century B.C., which, however, are leather[4]; eighteen specimens from the Dead Sea scrolls, generally considered to be of about the time of Christ; five specimens of about A.D. 150 from the nearby

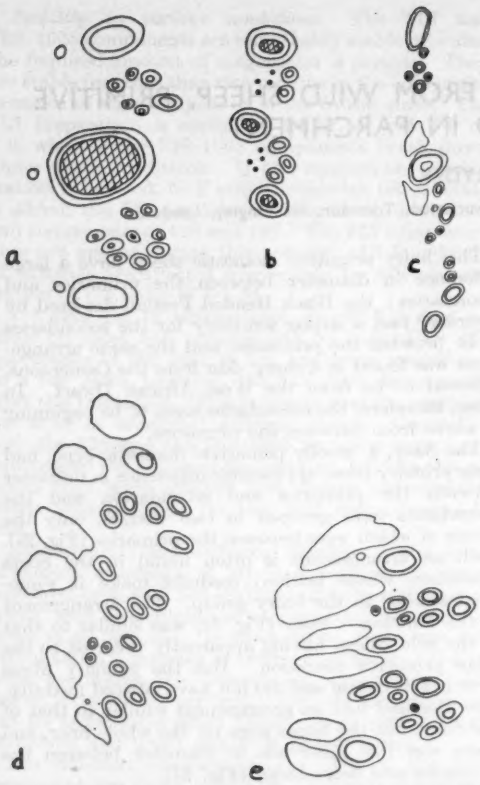


Fig. 2. Diagrams prepared from projection microscope tracings of follicle groups (ectal margin to right) from (*a*) goat; (*b*) Barbary lamb; (*c*) Mouflon × Soay; (*d*) Shetland; (*e*) Soay. Developing follicles in full black, latticed medullæ cross-hatched. Where visible, sebaceous glands have been indicated by irregular outlines and sweat glands by simple circles. Barbary lamb (× 82); others (× 41)

excavations at Murabba'at; and a sample of an English parchment dated 1692.

The histological structure of the follicles was remarkably well preserved and nuclei were often visible, although there had been some changes in staining reaction. The present description will, however, be restricted mainly to follicle grouping. This was perfect in only two Dead Sea scroll samples and two from Murabba'at, one from each of the three types of sheep found, namely, hairy, medium-woolled and fine-woolled, and a calf. All the samples showed traces of follicles, and as all but one had fibres, their type could usually be ascertained. The hairy type had large primaries with groups of small secondaries wedged between them (Fig. 3*a*). It is difficult to say whether this is from a sheep or a goat, but the secondary/primary ratio of about 4/1 suggests sheep rather than goat, which has a ratio of about 2/1. Three other samples, including one from Murabba'at, had both large and small follicles and were probably of this type.

The medium-woolled type (Fig. 4) had an arrangement similar to that of the Soay, with an appreciable difference in diameter between the primaries and secondaries, and a secondary/primary ratio of about 5/1. The fibres were non-medullated and had an irregular mosaic scale pattern, which in places approached the diagonal pattern characteristic of lustre (long-woolled) types; this is not found in the Soay. Five other scroll samples and one from Murabba'at had fibres of a similar diameter, and at least three had this diagonal scale pattern.

The fine-woolled type was most striking (Fig. 3*b*); it had large follicle groups with a secondary/primary ratio of more than 7/1. Such a high ratio and small fibre diameter are uncommon in modern British sheep, and this together with the characteristic scale pattern of fine wool suggests Merino type, which has been thought to originate in the Middle East. There were two other samples with fine fibres and the same scale pattern; a further four samples appeared to be coarser than this type, yet not as coarse as the medium type, and three samples, including one from Murabba'at, although apparently sheep, could not be classified. Two samples identified as calf, one from the scrolls and one from Murabba'at, were the only ones showing pigment.

The Aramaic documents have so far shown no clear groups; but traces of large follicles have been seen, as well as fine fibres, again suggesting a hairy type.

The English parchment of 1692 had fibres as fine as the fine type above, but the groups were smaller.

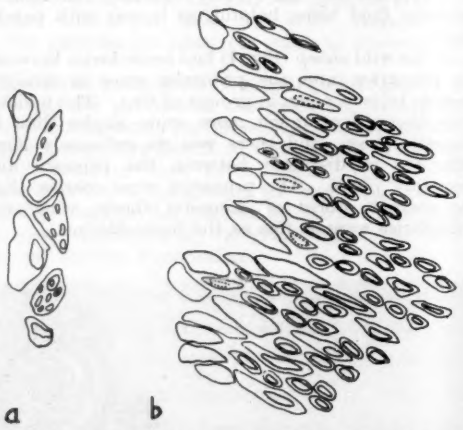


Fig. 3. Tracings of follicle groups in parchment from the Dead Sea scrolls: (*a*) hairy sheep or goat; (*b*) fine-woolled sheep (× 55)

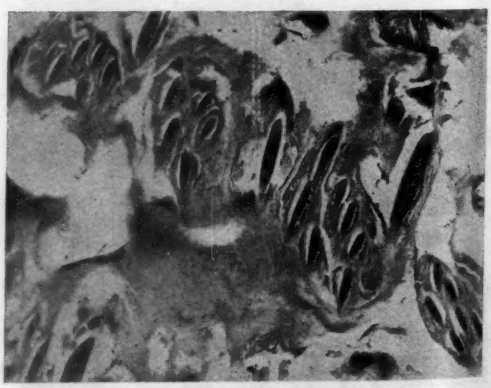


Fig. 4. Follicle group from medium-woolled sheep in parchment sample from Murabba'at (× 63)

Plug-like bodies were seen, suggesting either developing or shedding follicles; developing follicles would indicate a lamb. The fibres were finer than those of a modern day-old Romney lamb, and many of the follicles, but apparently none of the fibres, were pigmented.

It is hoped that these striking findings will stimulate the provision of material by libraries and museums, so that a more extensive study can be made, when the relation of these findings to the domestic evolution of sheep will be discussed.

I wish to thank the authorities of the British Museum (Natural History) and the Royal Scottish Museum for skin samples, Dr. A. T. Sandison for the material from a mummified cat, and Mr. W. N. Beesley for bringing a skin from the Cameroons. I am indebted to Prof. Donald Burton, Dr. R. Reed and Mr. J. B. Poole, of the Department of Leather Industries, The University, Leeds 2, for providing the material from the Dead Sea scrolls and Murabba'at and the other leather and parchment samples. My thanks are also due to Mr. G. R. Standley and Miss M. M. Vickers for technical assistance.

[1] Ryder, M. L., *J. Agric. Sci.*, **49**, 275 (1957).
[2] Sandison, A. T., *Stain Tech.*, **30**, 277 (1955).
[3] Duerden, J. E., *Trans. Roy. Soc. Edin.*, **59**, 763 (1939).
[4] Driver, G. R., "Aramaic Documents of the Fifth Century B.C." (Clarendon Press, Oxford, 1957).

SALT GLANDS IN MARINE REPTILES

By KNUT SCHMIDT-NIELSEN
Department of Zoology, Duke University, Durham, North Carolina
AND
RAGNAR FANGE
Zoophysiological Institute, University of Lund, Sweden

"SO they went up to the Mock Turtle, who looked at them with large eyes full of tears"[1]; "'for I am the Crocodile,' and he wept crocodile-tears to show it was quite true"[2]. Frequent literary references to a propensity for tear production in reptiles have not sufficiently challenged scientific imagination, and an interesting chapter in the physiology of excretion has remained unnoticed.

The finding that marine birds depend on a gland in the head rather than the kidney for the excretion of salt[3,4] suggested an investigation of their reptilian relatives, the marine turtles, crocodiles, snakes and lizards. These animals have maintained the terrestrial habit of air-breathing, and have an integument practically impermeable to salt and water. In these respects their physiology is essentially that of a terrestrial animal, but an important difference is that their food often has a high salt content, and the only drinking water available contains 3·5 per cent salt. Thus, their problems of osmo-regulation are comparable to those of marine birds.

Marine birds can tolerate the ingestion of sea water because the salt gland can excrete sodium chloride in a concentration almost twice as high as in sea water. Thus, marine birds do not suffer from the intake of sea water, in spite of the inability of their kidney to produce a urine more than about half as concentrated as sea water. The concentrating ability of the reptilian kidney is even lower, and marine reptiles could therefore be expected to have a mechanism for salt excretion similar to that in marine birds.

Marine reptiles. Reptiles with a marine habitat are found in four different orders: turtles, crocodiles, snakes and lizards.

Five species of turtles are strictly marine, going on land only to lay their eggs in the sand of tropical beaches. Their food consists of fish, some invertebrates, or seaweed. While the fish have a relatively low salt content, invertebrates as well as seaweed have the same osmotic concentration as sea water. These latter types of food, therefore, impose a heavy burden on the processes of osmo-regulation. Other turtles are found in brackish water, such as the diamond-back terrapin, *Malacolemys terrapin*. This animal is never found in truly fresh water[5].

One species of crocodile, the salt water crocodile (*Crocodylus porosus*), is estuarine in its habits, but has occasionally been found far out at sea in the Indian and Pacific Oceans. It is a carnivore, subsisting mainly on fish.

The sea-snakes, Hydrophidae, are common in the Indian Ocean. Some of the more primitive (*Laticauda*) lay their eggs on land, while the more specialized bear living young. They are all excellent swimmers and live on fish. Probably many other snakes are able to live in brackish water, an example being *Natrix sipedon compressicauda*, which is reported from brackish swamps in Florida[5].

One lizard, the Galapagos sea iguana (*Amblyrhynchus cristatus*), is well known for its appearance as well as its habits. It lives in the surf of the Galapagos Islands, where it climbs on rocks and eats seaweed exclusively[6]. A few other lizards also have been reported from a marine habitat[5].

Head glands. Several different kinds of head glands are found in the four reptilian orders mentioned. There are glands in the orbit of the eye (Harderian gland and lachrymal glands), glands opening into the nasal cavity (nasal glands), and glands opening into the mouth (salivary glands, poison glands, etc.).

In order to find whether marine reptiles are provided with salt glands we used two approaches, studies of (1) the histological structure of the head glands, and (2) the secretion obtained after a salt load, or after stimulation of the glands with the drug methacholine, a drug which stimulates glandular secretion in general.

Turtles have a large gland on each side of the head, located in the orbit of the eye. In the logger-

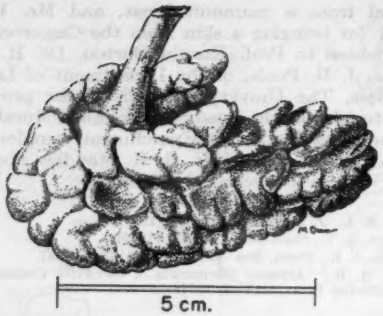


Fig. 1. Salt gland of the loggerhead turtle, *Caretta caretta*

head turtle, *Caretta caretta*, this gland (Fig. 1) is large, reddish-brown, and divided into about one hundred lobes separated by blood vessels and connective tissue. It connects to the exterior via a short, wide duct that opens in the posterior corner of the eye. The two glands weighed 60 gm. in a turtle of 130 kgm., which is 0·05 per cent of the body-weight. The relative weight of the gland was similar in the diamond-back terrapin. In the herring gull we have found the salt gland to be almost 0·1 per cent of the body-weight, and in the brown pelican 0·03 per cent.

The loggerhead gland consists of closely packed branching glandular tubules (Fig. 2) radiating from central ducts or canals in the lobes. This characteristic histological structure, which has previously been observed in the large 'lachrymal' gland of *Chelonia mydas*[7], is similar to the structure of the avian salt gland[8]. The structure of the terrapin gland is similar but less regular.

Crocodiles have well-developed nasal and Harderian glands[9,10], but the functions of these glands are not known. The nasal gland in *Crocodylus porosus* has been described as a rather large acinous gland[11].

Several sea-snakes, for example *Enhydris*, have a well-developed nasal gland[12]. It is, however, missing in one sea-snake, *Pelamis*. According to our own observations on a sea-snake (*Hydrophis ornata*) there is a gland above the eye with a histological structure resembling that of the salt gland of the terrapin. The gland consists of closely packed glandular tubules.

The marine iguana has a large nasal gland which in a specimen of 490 gm. weighed about 300 mgm. (0·06 per cent of the body-weight). Histologically the structure of this gland resembles that of the avian salt gland. The gland opens, via a short duct, into the lateral nasal cavity, which seemingly can function as a reservoir for the secretion. A ridge prevents the secretion from flowing back into the posterior part of the nasal cavity, from where it would drain into the œsophagus. On the other hand, a sudden expiration of air would force the liquid out through the nostril.

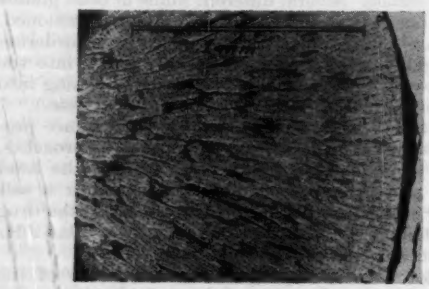


Fig. 2. Microscopic structure of salt gland of the loggerhead turtle

Secretion from salt glands. Marine turtles, when given an injection of hypertonic sodium chloride, secrete a highly concentrated salt solution from the orbital salt gland. This we could demonstrate in the diamond-back terrapin (*Malacolemys terrapin*), a brackish water turtle from Florida, which, when given 10 ml. of 10 per cent sodium chloride per kgm. body-weight, secreted a fluid which appeared as 'tears' in the eye. The sodium concentration of this fluid was 616–784 m.equiv. sodium/l. This salt concentration is well above that of sea water (about 500 m.equiv. sodium/l.).

In a large specimen of the completely marine loggerhead turtle, the duct of the salt gland was easily cannulated, and it could be established with certainty that the secretion came from this particular gland. Samples of secretion obtained after salt load and stimulation with methacholine contained 732–878 m.equiv. sodium/l., 810–992 m.equiv. chlorine/l. and 18–31 m.equiv. potassium/l. These concentrations are similar to those produced by the salt gland of marine birds.

The fact that turtles cry tears when they go on shore to lay their eggs has received much attention. The anthropomorphic interpretation that the tears are induced by the pain of egg-laying has been rejected by naturalists, who instead have speculated that they may serve to keep the eyes from drying out, or to keep the sand out. The latter obviously is not compatible with the descriptions such as that given by Carr[13]: "The curious 'crying' by nesting females (apparently a device to keep the eyes washed free of sand) has been noted in other species. The present turtle began secreting copious tears shortly after she left the water, and these continued to flow as the nest was dug. By the time she began to lay, her eyes were closed and plastered over with tear-soaked sand and the effect was doleful in the extreme".

It may instead be suggested that the turtle tears are produced in response to the osmotic load of living and feeding in the sea, and that their major role may be osmo-regulatory.

We had no opportunity to work with living specimens of the salt-water crocodile, nor with living sea-snakes. One water snake, the Mangrove water snake (*Natrix sipedon compressicauda*), which is a sub-species of the common water snake, showed no secretion from head glands when given a salt load. Stimulation with methacholine gave only slight secretion from mucous glands. We did not find any glands in the head of *N. s. compressicauda* that could be interpreted anatomically as salt glands.

The marine iguana has repeatedly been observed to blow out "a thin shower of water vapour" from the nostrils[14]. We induced secretion in two animals that had been kept in captivity for three months, by the injection of sodium chloride (10 ml. of 10 per cent solution per kgm. body-weight) and methacholine. The secreted fluid was ejected from the nostrils as fine drops.

When picking up a lizard one of us was hit in the face and eyes with a jet of droplets that had a salty taste. This rather subjective observation of a high salt content was followed by a more accurate

determination with a flame photometer, which showed a concentration of 840 m.equiv. sodium/l. in the secreted fluid.

Since the Galapagos lizard eats only seaweed which has a salt concentration similar to that of sea water, it is not only useful, but also a necessity, for it to have a mechanism for excretion of the salts in a high concentration.

In summary, we can conclude from observations described in this article that the combination of circumstantial (histological) and direct evidence (secretion of salt) indicates that an extrarenal mechanism for salt excretion is present probably in all four orders of marine reptiles, turtles, crocodiles, snakes and lizards. The secretion derives from head glands which resemble the salt glands of marine birds, both in structure and function. The kidney of birds and reptiles cannot produce a urine with high salt concentrations, and one can assume that the extrarenal mechanism for salt excretion is a necessity for successful adaptation to a marine habitat in all sauropsidians.

We wish to acknowledge the generous help and co-operation of Drs. Doris M. Cochran and H. Friedmann (Smithsonian Institution), Mr. R. Hathaway (Florida State University), Dr. R. C. Miller (California Academy of Sciences), Dr. J. A. Oliver (New York Zoological Society), and Mr. F. G. Wood (Marineland Research Laboratory, Florida). The work was supported by Grant *H*-2228 from the National Institutes of Health.

[1] Carroll, Lewis, "Alice's Adventures in Wonderland".
[2] Kipling, Rudyard, "Just So Stories".
[3] Schmidt-Nielsen, K., and Sladen, W. J. L., *Nature*, **181**, 1217 (1958).
[4] Schmidt-Nielsen, K., Jörgensen, C. B., and Osaki, H., *Amer. J. Physiol.*, **193**, 101 (1958).
[5] Neill, W. T., *Bull. Mar. Sci. Gulf and Caribbean*, **8**, 1 (1958).
[6] Darwin, Charles, "The Voyage of the Beagle", 371 (Dutton, New York, 1950).
[7] Peters, A., *Arch. Mikr. Anat.*, **36**, 192 (1890).
[8] Fänge, R., Schmidt-Nielsen, K., and Osaki, H., *Biol. Bull.* (In the press).
[9] Reese, A. M., *Biol. Gen.*, **1**, 482 (1925).
[10] Bellairs, A. D'A., and Shute, C. C. D., *J. Anat.*, **7**, 367 (1953).
[11] Rose, C., *Anat. Anz.*, **8**, 745 (1893).
[12] Kathariner, L., *Zool. Jb.*, **13**, 415 (1900).
[13] Carr, Archie, "Handbook of Turtles", 409 (Cornell University Press, Ithaca, 1952).
[14] Beebe, W., "Galapagos, World's End", 119 (Putnam, New York and London, 1924).

INFRA-RED SPECTRA OF A COALIFICATION SERIES FROM CELLULOSE AND LIGNIN TO ANTHRACITE

By Prof. C. R. KINNEY and E. I. DOUCETTE*

Department of Fuel Technology, Pennsylvania State University, University Park, Pa.

THE chemical processes involved in the transformation of the woody parts of plants into coal are not well understood. For the purpose of gaining insight into these processes, infra-red spectra of a series of coal-source materials from cellulose, lignin and wood through peat wood and a brown coal to typical bright coals of increasing rank have been compared. Significant changes in the spectra may be observed which reflect important chemical changes in the coalification series.

Characteristic spectra are reproduced in Fig. 1, and the sources of the samples are given in Table 1. The spectra were made using a 0·5 per cent concentration of finely ground sample in each case, dispersed in pressed potassium bromide plates. Consequently, the intensities of the absorptions should be only relative. Since the coals of low rank are harder to grind, the sloping background absorption in the region 2–6μ (Fig. 1) indicates that these samples were not ground finely enough to prevent some dispersion of the radiation at these wavelengths. Although this tends to decrease the relative intensity of the peaks in this region of these samples, the amount is not considered serious for making comparisons.

The strong absorption at about 8·5–10μ in the spectra of α-cellulose, holocellulose, lignin, fresh wood and peat wood (estimated to be 20 million years old) in Fig. 1 is unquestionably due to the stretching vibrations of carbon–oxygen bonds of various oxygen-containing groups which these materials are known to possess, particularly hydroxyl and ether linkages. On passing from peat to brown coal and to coals of higher rank there is a marked decrease in this absorption. Many of the coals contain considerable amounts of siliceous mineral matter which also absorbs at 9·7μ. In the coal spectra, the decrease in absorption between 8·5 and 10μ is accompanied by a corresponding decrease in the hydroxyl absorptions at 2·9μ and aliphatic carbon–hydrogen absorptions at 3·4–3·5μ. These changes accompany the disappearance of cellulose and lignin as such on passing from wood to coal, and must be due primarily to the loss of hydroxyl groups by the elimination of water molecules.

Table 1. Identification of Infra-red Samples

Sample*	Source
Meta-anthracite	Rhode Island†
Anthracite	Pennsylvania‡
Low-volatile bit.	Pocahontas, W. Va.‡
Med.-volatile bit.	*B* Seam, Kentucky‡
High-volatile *A* bit.	Pittsburgh Seam, Pa.‡
High-volatile *B* bit.	No. 6 Seam, Illinois‡
Sub-bituminous *B*	Rosebud Co., Montana§
Lignite	Bowman Co., North Dakota§
Brown coal	Washington, D.C.‡
Peat wood	Peat Bog, Ohio‡
Fresh wood	Aspen Wood‖
Sulphite lignin	Aspen Wood‖
Holocellulose	Aspen Wood‖
α-Cellulose	Aspen Wood‖

* ASTM rank.
† Obtained from Dr. H. J. Donald, then in the Department of Fuel Technology, Pennsylvania State University.
‡ Obtained from Dr. W. Spackman, Department of Geology, Pennsylvania State University.
§ Obtained from Dr. A. Traverse, then with Bureau of Mines, Grand Forks, North Dakota.
‖ Obtained from Forest Products Laboratory, Madison, Wisconsin.

* Present address: Bell Telephone Co., Murray Hill, New Jersey.

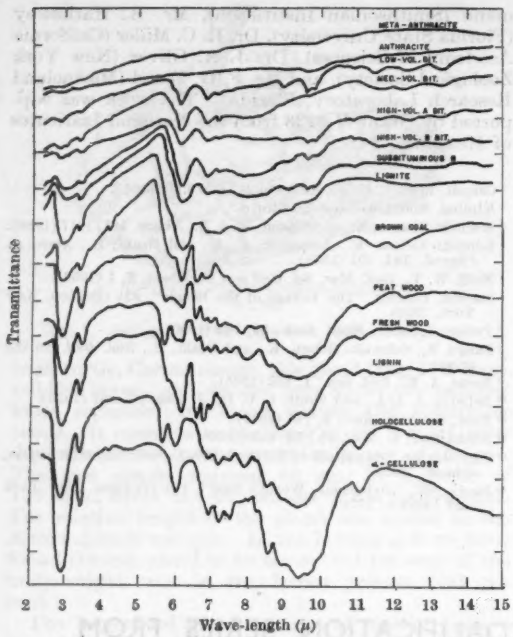


Fig. 1. Infra-red spectra of coal-forming substances and characteristic coals

Another absorption that is largely eliminated in the same early stages of coalification is the carbonyl band at 5·8μ in the spectra of holocellulose, wood and, to a lesser extent, in lignin and peat wood. In the spectra of the brown coal, this band is markedly reduced, and in the higher rank coals is practically eliminated. Adams and Pitts[1] find that bituminous coals protected from air oxidation have no band in this region. The 5·8μ band in the spectra of holocellulose and wood is believed to be due to acetyl groups which these materials contain and to the small amount of carbonyl present in the lignin structure. In peat it may be presumed that all of the acetyl groups would soon be removed by hydrolysis. This was confirmed by boiling a sample of wood with water for a number of hours and the 5·8μ absorption was reduced, similar to that in the peat spectrum. Also, the 6·0 and 7·9μ absorptions present in the fresh wood and holocellulose spectra were eliminated, with the result that the spectrum of the boiled wood was very similar to that of peat wood.

The broad absorption in the brown coal, centring at about 8μ, diminishes in lignite and coals of higher rank. This is accompanied by a corresponding loss in hydroxyl absorption at 2·9μ, and is no doubt due to the loss of phenolic hydroxyl groups as coalification progresses[2]. Since aliphatic carbon–hydrogen absorption at 3·4 to 3·5μ does not appear to decrease in intensity at the same time, it seems probable that the phenolic hydroxyl groups are not split out as water with aliphatic hydrogen atoms. Aromatic ether linkages absorb strongly at about 8μ and a part of the decrease in absorption in this region, as brown coal undergoes further coalification, may be due to the elimination of ether linkages.

The development of the strong band at 6·2μ in coal spectra (Fig. 1) is of particular interest. This is the region of double-bond stretching vibrations, particularly C=C bonds, and in coal spectra this absorption has been assigned to aromatic structures[3–5]. Other authors[6–8] have proposed that at least a part of this absorption is due to groups containing oxygen, particularly hydrogen-bonded quinones. The spectra of cellulose, holocellulose, and wood all show an absorption at 6·0μ, which may be due to hydrogen-bonded carbonyl groups, but this band disappears at the peat stage with no corresponding increase in the 6·2μ absorption. Consequently, it appears that the cellulosic constituents of wood are not the source of this absorption.

Lignin, on the other hand, has a strong 6·2μ band, which is observed in the spectra of wood, peat and the various coals to the semi-anthracite rank with but little diminution of intensity from wood to semi-anthracite. In the spectrum of lignin, the 6·2μ band is undoubtedly due to the benzene rings which this material contains. The 6·2μ band in lignin is accompanied by a second band at 6·6μ, as is frequently observed with benzene derivatives. These two bands of nearly equal intensity may be observed in the spectra of wood, peat and the brown coal, but in the lignite spectrum the absorption at 6·6μ is greatly reduced and is completely absent in the spectra of higher rank coals. This loss in absorption at 6·6μ on passing from low-rank to high-rank lignites was observed with three additional samples of the former and four of the latter, suggesting that the 6·6μ band disappears in the early stages of coalification. Brown[9] has also pointed out that the 6·6μ band is generally weak or absent in spectra of known condensed aromatic hydrocarbons containing more than three rings. This band is also absent from spectra of coal tar pitches[5], which are composed of mainly large condensed aromatic structures. Therefore, it seems probable that the cause of the disappearance of the 6·6μ band is the condensation of benzenoid derivatives characteristic of lignin into polynuclear fused-ring aromatic structures characteristic of coals[9].

The bands at 6·9 and 7·2μ in the coal spectra, together with bands at 3·4–3·5μ, have been assigned to aliphatic carbon–hydrogen groups[3,4,6]. The persistence of these bands throughout the bituminous range of coals shows that this structural element is one of the last to disappear. The absence of these bands in the spectra of anthracites may be due more to their obliteration by a general electronic type of absorption[3] rather than to a complete elimination of these structures.

A comparison of the spectra of the coalification series from cellulose and lignin to anthracite gives an insight into the chemical changes that occur during coalification. Of particular interest are the loss of oxygen and the development of aromatic absorption characteristic of condensed polynuclear aromatic structures.

[1] Adams, W. N., and Pitts, G. J., *Fuel*, **34**, 383 (1955).
[2] Blom, L., Edelhausen, L., and van Krevelen, D.W., *Fuel*, **36**, 135 (1957).
[3] van Vucht, H. A., Rietveld, B. J., and van Krevelen, D. W., *Fuel*, **34**, 50 (1955).
[4] Bergman, G., Huck, G., Karwell, J., and Luther, H., *Brennstoff-Chemie*, **38**, 193 (1957).
[5] Unpublished work. See also Monnot, G. A., and Morel, P., *Applied Spectroscopy*, **9**, 180 (1955).
[6] Brown, J. K., *J. Chem. Soc.*, 744 (1955).
[7] Friedel, R. A., and Queiser, J. A., *Anal. Chem.*, **28**, 22 (1956).
[8] Hadzi, D., *Fuel*, **35**, 77 (1956).
[9] Howard, H. C., *Indust. Eng. Chem.*, **44**, 1083 (1952).

LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. No notice is taken of anonymous communications.

Undergraduate Academic Record of Fellows of the Royal Society

At the present time the main criterion for the award of grants for postgraduate research seems to be the candidates' undergraduate academic record. As a preliminary attempt to measure the validity of this practice, we have examined the published class of degree gained by current Fellows of the Royal Society who received their first degree from Oxford or Cambridge. Our results are given in Tables 1 and 2. Oxford and Cambridge are listed separately, and in each case the results of those who graduated during the period 1920–39 are given as well as the results for the group as a whole. For the Oxford group the result given is always that gained in the Final Honours School; for the Cambridge group the results given in Table 1 are of the last Tripos taken irrespective of whether it was Part 1 or Part 2, while Table 2 gives the results of an earlier Tripos if one was taken. The figures in brackets represent the actual totals on which the percentages are based.

Table 1. Results in Final Honours School (Oxford), or Last Tripos (Cambridge)

Cambridge					Oxford				
Class	1920–1939	Per cent	1886–present	Per cent	Class	1920–1939	Per cent	1907–present	Per cent
1	(70)	76·9	(139)	78·5	1	(30)	96·8	(36)	97·3
2	(19)	20·9	(34)	19·2	2	(1)	3·2	(1)	2·7
3	(2)	2·2	(4)	2·3	3	—	—	—	—

Table 2. Cambridge Tripos Results for Part 1 and Part 2

Class		Cambridge			
Part 1	Part 2	1920–1939	Per cent	1886–present	Per cent
1	1	(50)	54·9	(95)	53·7
2 or 3	1	(8)	8·8	(14)	7·9
1	2 or 3	(10)	11·0	(20)	11·3
2 or 3	2 or 3	(7)	7·7	(9)	5·1
1		(12)	13·2	(30)	16·9
2 or 3		(4)	4·4	(9)	5·1

If we assume that Fellows of the Royal Society have demonstrated outstanding research ability and that examination standards at Oxford and Cambridge have remained relatively constant (at least during the period 1920–39), and if academic records are a good predictive criterion of research ability, we should expect to find a negligible proportion within our criterion group who gained anything other than first class degrees as undergraduates. Therefore our first finding (Table 1) that approximately 1 in 4 of the group who graduated from Cambridge during the period 1920–39 gained a second or third in their final examination, and that (Table 2) approximately 1 in 3 gained either a second or third at some time during their undergraduate careers seems surprising. Our other principal finding, namely, that the Oxford group appear to have better undergraduate records than the Cambridge group, seems open to at least two interpretations: (1) that the Oxford examination is actually a more valid index of potential research ability than is the Cambridge one; and (2) that the standards of the Oxford examinations are relatively lower than they are at Cambridge. A third possible interpretation, that the sample is inadequate to make the difference significant, is unlikely at the 0·5 per cent level of confidence using the χ^2.

Although the present data do not enable us to state the general relation between undergraduate academic achievement and research ability, we believe that considerable doubt has been cast on the use of degree class alone as a criterion for the selection of research students. In order that a more precise assessment may be made, we hope to carry out the following further investigations: (1) compare the proportion of degree classes gained by Fellows of the Royal Society with the proportion gained by the population of research students at Oxford and Cambridge during the relevant period; (2) extend the list of Fellows to include those who graduated from universities other than Oxford and Cambridge; and (3) examine other criterion groups, such, for example, as D.Sc.'s.

The encouragement of Profs. F. G. Young and O. L. Zangwill throughout this inquiry is gratefully acknowledged.

C. Gross
L. Hudson

Psychological Laboratory,
Cambridge.
July 16.

Effect of Air Drag on the Orbit of an Earth Satellite

It has been shown[1] that the first-order effect of the oblateness of the Earth can be simply incorporated in the standard analysis of the effect of air drag on a satellite orbit. However, King-Hele and Leslie[2] ignore this correction in a comparison of theory and observation on the orbit of *Sputnik* 2 (1957β) in favour of terms of higher order in the eccentricity.

There appear to be two distinct problems in the fitting of the observational data, as typified by the

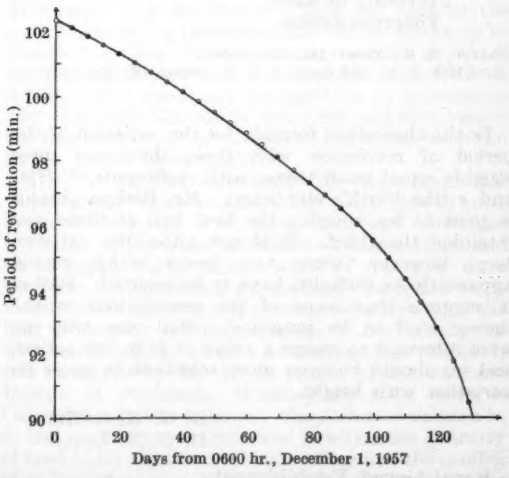


Fig. 1. Period of *Sputnik* 2: —, observed; O, calculated

prediction of the orbital period over a range of the order of months on one hand and by the day-to-day variation in the rate of increase of the periodic time on the other. This second problem obviously requires a consideration of effects which are ignored in the customary analysis, of which the tidal influences of the Moon may well be one. However, the variations of this second type appear to be grouped about a mean behaviour consistent with the simple analysis. Thus Fig. 1 shows a comparison between the observed orbital period and that calculated on the relation given by me[1] over the period December 1–April 10. Here only first-order terms in e_0 are retained, but a correction for oblateness is included. The value of t_n was taken as 134·4 days and the scale-height was estimated at 33·5 nautical miles. The fit is decidedly better than that of King-Hele and Leslie, the only noticeable departure of the calculated values occurring in January when observations indicated a change in the effective drag cross-section. It should be noted that this agreement has not been obtained by an arbitrary assignment of parameters, since t_n is critically determined by the time of final fall and the only assignable parameter is the scale-height H. Undoubtedly the assumption of constant scale-height is violated in practice; but unless the agreement is purely fortuitous, it would appear that it varies little between 112 nautical miles and 95 nautical miles above the surface of the Earth. There is, however, some indication in the orbital data of a slight decrease in the value at lower levels.

It would seem that for prediction 'in the large' an analysis correct to the first order in e_0 but including a correction for the oblateness of the Earth is entirely adequate. This is further borne out by the fact that the failure of the asymptotic expansions of the Bessel functions at small values of the eccentricity is illusory, since the instability of the 'circular' form implies a non-zero minimum value of e. The second problem, that of accounting for the variations in the very sensitive rate of increase of periodic time, quite obviously requires considerably wider physical assumptions and is consequently beyond the scope of the present analysis.

D. G. Parkyn

Department of Mathematics,
University of Natal,
Pietermaritzburg.

[1] Parkyn, D. G., *Nature*, **181**, 1156 (1958).
[2] King-Hele, D. G., and Leslie, D. C. M., *Nature*, **181**, 1761 (1958).

In the theoretical formula for the variation of the period of revolution with time, there are three roughly equal small terms, with coefficients e^2, H/a_0 and ε (the Earth's ellipticity). Mr. Parkyn obtains a good fit by ignoring the first two of these and retaining the third. It is not altogether satisfactory, however, when two terms which should apparently be included have to be omitted. Rather it suggests that some of the assumptions in the theory need to be improved; that was why we were reluctant to assign a value of H in our article, and we should be even more reluctant to guess its variation with height.

D. G. King-Hele
D. C. M. Leslie

Royal Aircraft Establishment,
Farnborough, Hants.

The Fundamental Molecular Event in Muscular Contraction

Astbury[1,2] suggested that the fundamental event in the contraction of muscle might involve a characteristic of the keratin–myosin–epidermin–fibrinogen group of fibrous proteins to which myosin belongs, namely, the folding or collapsing of polypeptide chains from the α-configuration into the supercontracted state. While it has been shown that isolated myosin and actomyosin supercontracts after reasonably mild chemical or physical treatment[2,3], no X-ray evidence has been forthcoming so far to show that the shrinkage of actomyosin gels after treatment with adenosine triphosphate is accompanied by a corresponding configurational change[4]. Moreover, there is the difficulty of reconciling this approach with recent concepts of a two-phase actin–myosin system shortening by close-range[5] or long-range[6] interaction of filaments coiling or sliding over one another in a regular stepwise fashion.

The demonstration by Astbury, Beighton and Weibull[7], in diffraction diagrams of bacterial flagella, of X-ray features characteristic of the supercontracted state (the 'cross-β' configuration[8]) side by side with an α-pattern having analogies with that of muscle suggests that the simplest molecular expression, of flagellar motility in any event, may be a rhythmic interchange between the α-state and a shorter supercontracted form. Recently, the discovery by Rudall[9] of a transversely folded protein in the egg-stalk of the lace-wing fly *Chrysopa* has much emphasized the possibility that there may commonly exist in Nature (as opposed to mechanical or chemical artefacts) a well-defined protein state in which the polypeptide chains lie in long regular folds[10].

We now report that the synæresis of actomyosin on addition of adenosine triphosphate is accompanied by X-ray indications of a transformation of a proportion of the protein into the supercontracted state.

The actomyosin used was extracted from the finely crushed dorsal muscle of fish by buffered alkaline potassium chloride and precipitated by lowering the ionic strength. After washing three times, with interspersed centrifugation, the actomyosin formed transparent thixotropic gels, still intensely reactive to adenosine triphosphate solutions[11] and capable of being dried on water-repellent glass blocks to form thin transparent films. The unsynæresed films could easily be stretched in moist air to give well-oriented α-diagrams characterized by the familiar meridional reflexion at about 5·1 A. After synæresis with adenosine triphosphate, the resultant opaque brittle films were difficult to handle, and the diffraction patterns of such unpromising material appeared to show only a vague sharpening on the meridian. By washing out the reactants immediately after addition of adenosine triphosphate, however, the partly contracted gels retained a certain capacity to stretch after drying, and such specimens could be sufficiently oriented to illustrate changes in the diffraction pattern.

Fig. 1 shows the diffraction pattern of a film prepared as just described of actomyosin from the dorsal muscle of the ruffe, *Acerina vulgaris*. The 'cross-β' configuration shows up as a faint preferred orientation at 4·65 A. on the meridian. The conditions for the appearance of an X-ray pattern indicating supercontraction in actomyosin appear to be very critical;

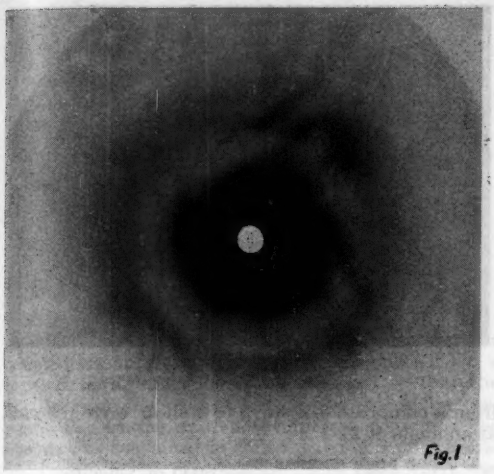


Fig. 1. Actomyosin from *Acerina vulgaris* partly contracted with adenosine triphosphate. Thin films stretched about 50 per cent in moist air and photographed with the X-ray beam parallel to the films

this is understandable if the circumstances of protein chain-folding are such as to obstruct orientation by stretching.

A detailed account of this work will appear in due course. I am indebted to Prof. W. T. Astbury for his encouragement, to Dr. K. M. Rudall for advice, and to Mr. A. Millard for reproducing the X-ray diffraction photograph shown in Fig. 1.

F. G. E. Pautard

Department of Biomolecular Structure,
The University,
Leeds 2.

[1] Astbury, W. T., *Proc. Roy. Soc.*, B, **134**, 303 (1947) (Croonian Lecture, 1945).
[2] Astbury, W. T., and Dickinson, S., *Proc. Roy. Soc.*, B, **129**, 307 (1940).
[3] Rudall, K. M., "Progress in Biophysics", **1**, 39, edit. by Butler, J. A. V., and Randall, J. T. (Butterworth-Springer, Ltd., London, 1950).
[4] Astbury, W. T., Reed, R., and Spark, L. C., *Biochem. J.*, **43**, 282 (1948).
[5] Spiro, D., *Exp. Cell. Res.*, **10**, 562 (1956).
[6] Hanson, J., and Huxley, H. E., *Symp. Soc. Exp. Biol.*, **9**, 228 (1955).
[7] Astbury, W. T., Beighton, E., and Weibull, C., *Symp. Soc. Exp. Biol.*, **9**, 282 (1955).
[8] Rudall, K. M., Symp. on Fibrous Proteins, *J. Soc. Dyers and Colourists* (1946); "Advances in Protein Chemistry", **7**, 255 (1952).
[9] Parker, K. D., and Rudall, K. M., *Nature*, **179**, 905 (1957).
[10] Astbury, W. T., Introduction to the section on Macromolecules, Faraday Society General Discussion on "Configurations and Interactions of Macromolecules and Liquid Crystals", Leeds, April 1958 (in the press).
[11] Szent-Györgyi, A., "Chemistry of Muscular Contraction" (Academic Press, New York, 1951).

Calibration of a Calorimeter: a Minor Peaceful Use for Plutonium

Calorimeters are often calibrated by generating a known electric current within them and observing the deflexion of the measuring instrument when the system is in a steady state. This method may suffer from the disadvantage that heat is lost along the leads through which the calibrating current is introduced. The loss of heat can be reduced by passing the leads through a block of metal maintained at the experimental temperature, though the design of the calorimeter and its water-bath may make this procedure difficult. One can also calculate the loss of heat along the heater leads, but it is not desirable to rely exclusively on estimates that cannot be verified by experiments. One alternative is to calibrate the heater against some thermochemically convenient reaction, for example, the latent heat of vaporization of a liquid[1], or the neutralization of a strong acid by a strong base[2]. These methods have their own disadvantages, and it was therefore decided to use a different method of calibration, either of the calorimeter itself or of the heater used to calibrate it.

This method, which involves putting into the calorimeter a radioactive substance in equilibrium with its disintegration products, in a radiation-absorbing container, was first used by Boivinet and Calvet in 1954[3]. They inserted into their calorimeter a platinum and gold needle containing 10 mgm. radium in equilibrium with its disintegration products, which produced about 95 mcal./hr. The radium needle was electrically calibrated in an adiabatic calorimeter in which the heat conduction along the heater leads was said to be negligible[4].

Radium has certain disadvantages for this purpose: first, radium-226 and its decay products are strong γ-emitters, which is not conducive to ease of handling in a very small calorimeter. Secondly, unless the percentage of radium-228 in the sample of radium-226 is known, theoretical estimates of the energy output of the sample cannot be precise. Plutonium-239 does not suffer from these drawbacks and has the additional advantage that its only decay product, uranium-235, is very stable, having a half-life of $7{\cdot}07 \times 10^8$ years. Plutonium-239 was obtained by irradiating uranium-238 with slow neutrons in BEPO at the Atomic Energy Research Establishment, Harwell. The following reactions occur:

$$^{238}\mathrm{U}(n,\gamma)^{239}\mathrm{U} \xrightarrow{\beta} {}^{239}\mathrm{Np} \xrightarrow{\beta} {}^{239}\mathrm{Pu}(n,\gamma)^{240}\mathrm{Pu}(n,\gamma)^{241}\mathrm{Pu}$$
$$\downarrow (n,2n)$$
$$^{238}\mathrm{Pu}$$

The amounts of plutonium-238 and americium-241, plutonium-240 and plutonium-241 were, respectively, 0·3 per cent (by activity), 0·977 per cent and about 0·016 per cent. According to Asaro and Perlman[5], the total disintegration energy of plutonium-239 is 5·235 MeV./nucleus and the same value can be deduced from the earlier data of Gol'din, Tret'yakov and Novikova[6]. 99·6 per cent of the γ-rays emitted by plutonium-239 can be shown to be absorbed by the platinum/iridium needles in which the plutonium (as PuO_2) is contained. As 1 MeV. = $3{\cdot}829 \times 10^{-11}$ mcal. and the half-lives of plutonium-239, -240 and -238 are 24,360, 6,580 and 89·6 years[5], the heat output of the plutonium oxide can be shown to be 1·4891 mcal./mgm./hr.

The two needles containing the plutonium oxide were standard radium needles, external length 30 mm., external diameter 1·65 mm. and wall thickness 0·5 mm. They were believed to contain 41·58 and 49·66 mgm. PuO_2, though some of this may have been lost during sealing the needles. Using the value 1·4891 mcal./mgm./hr., a theoretical estimate of the heat production of the needles can be made (Table 1). The actual energy output of the needles (Table 1) was measured by Dr. W. B. Mann, of the National Bureau of Standards, Washington, D.C., using a Callendar radiation balance[7]. The radiation balance is, in fact, in the microcalorimeter in which the quantity of heat to be measured is balanced against the cooling from Peltier couples, in such a way that no corrections are needed for the Joule heating in the Peltier couples

Table 1. Energy Output of PuO_2 in mcal./hr.

Needle	Calculated	Measured (Mann)
1	61·92	62·26 ± 0·35
2	73·95	74·48 ± 0·35
1 ÷ 2 (measured)	0·8326 ± 0·0043 (Mann)	0·8292 ± 0·0089 (Rothschild)

and leads. This method of measuring heat production does not have the disadvantages mentioned earlier.

Using a modified Clarke–Rothschild calorimeter[2], volume 2·4 ml., for which the plutonium oxide needles were made, I obtained a value for the ratio needle 1/needle 2 of 0·829, as compared with Dr. Mann's value of 0·833. The difference, of about 0·6 per cent, between the calculated and observed energy outputs may be due to an error in the value taken for the half-life of plutonium-239, or to the sample not being pure PuO_2, or to the amount of americium-241 present.

I am greatly indebted to the U.S. National Bureau of Standards and, in particular, to Dr. W. B. Mann, for measuring the heat output of the plutonium needles; and to Dr. G. B. Cook, of the Isotope Division, Atomic Energy Research Establishment, Harwell, for much assistance and advice. This work is supported by the Medical Research Council.

Rothschild

Department of Zoology,
University of Cambridge.

[1] Calvet, E., *C.R. Acad. Sci., Paris*, **236**, 486 (1953).
[2] Clarke, E. W., and Rothschild, Lord, *Proc. Roy. Soc.*, B, **147**, 316 (1957).
[3] Boivinet, P., and Calvet, E., *C.R. Acad. Sci., Paris*, **238**, 1995 (1954).
[4] Calvet, E., and Prat, H., "Microcalorimétrie" (Masson et Cie., Paris, 1956).
[5] Asaro, F., and Perlman, I., *Rev. Mod. Phys.*, **29**, 831 (1957).
[6] Gol'din, L. L., Tret'yakov, E. F., and Novikova, G. I., Conf. Acad. Sci. U.S.S.R. on the Peaceful Uses of Atomic Energy, 226–250 (U.C.R.L. Trans. 242) (1955).
[7] Mann, W. B., *J. Res. Nat. Bur. Stand.*, **52**, 177 (1954a); **53**, 277 (1954b). Mann, W. B., and Seliger, H. H., *Nat. Bur. Stand. Circ.*, 594 (1958).

Visualization of Mode Conversion of an Ultrasonic Beam in Fused Quartz

In investigating methods of bonding transducers to fused quartz for use in delay lines, it is very useful to be able to see how the ultrasonic beam travels through the quartz.

To visualize the paths of such beams, we have employed the optical system described by Sven Johnson[1], which makes use of the following two principles: (1) a diffraction grating is produced in the quartz by the periodic variations of density in the path of the ultrasonic beam[2]; if the quartz be viewed in a schlieren system, only the vibrating portions of the quartz should be visible; (2) in the regions of high ultrasonic strain the quartz becomes birefringent; thus, placing the quartz between crossed 'Polaroids' will cut out the effect of scattered light without blocking the ultrasonic field from view.

Fig. 1 is a photograph, taken by this method, which gives a vivid illustration of the phenomenon of mode conversion on reflexion.

On reflexion of a compressional wave at a plane interface between a solid and air, both compressional and shear waves will be reflected. Arenberg[3] has calculated that, in fused quartz at an angle of incidence of 44·8°, there will be complete mode conversion

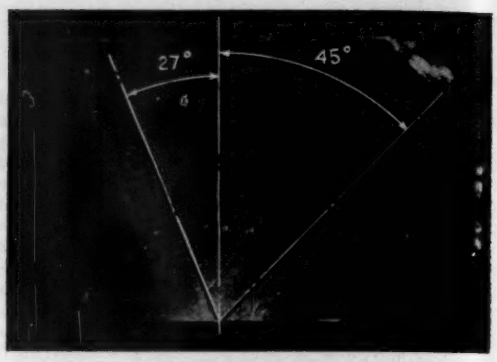


Fig. 1

from a compressional wave to a shear wave reflected at an angle of 27·2°:

$$\left(\frac{\text{sine angle incidence}}{\text{sine angle reflexion}} = \text{ratio of velocities}\right)$$

An *X*-cut quartz crystal, affixed to the Heraeus quartz blank by an indium bond, is excited at its resonant frequency of 8·5 Mc./s., thus injecting the compressional wave into the quartz (which has been machined to give the required angle of incidence). Fig. 1 shows the beam reflected at an angle of 27° and no sign of any reflexion at 44·8°. Some standing waves in the ultrasonic field are visible. Multiple reflexions have been reduced by absorbing material on the faces of the blank.

Acknowledgments are made to Mr. K. P. Osmond for the preparation of the bond and to Mr. A. Allen, chief development engineer of Cossor Radar and Electronics, Ltd., for permission to publish this communication.

V. J. Hammond
R. Carter

Research Department,
Cossor Radar and Electronics, Ltd.,
Highbury, London, N.5.
July 18.

[1] Johnson, Sven, D.S.I.R. Photostat PB.14122 of Andersen Lab. Report (1953).
[2] Bergmann, L., "Der Ultraschall" (S. Hirzel, Stuttgart, 1954).
[3] Arenberg, D. L., Report 932, M.I.T. Radiation Laboratory (1946).

Scattering of Microwave Radiation by a Plasma Column

The purpose of this communication is to describe experiments performed to determine the angular distribution of the electromagnetic radiation scattered by the positive column of a mercury discharge under the resonant conditions which have been previously investigated[1,2]. The experiments reported here prove the multiple order of the three largest resonances shown in Fig. 1 to be dipole.

The conditions for production of the series of resonances in the scattered radiation are that (1) the *E* vector of the incident radiation be perpendicular to the axis of the plasma column, (2) the diameter of the column be small compared to the wave-length of the radiation, and (3) the frequency of the radiation be in the vicinity of the so-called plasma frequency.

The following experimental arrangements were made in order to satisfy the foregoing conditions.

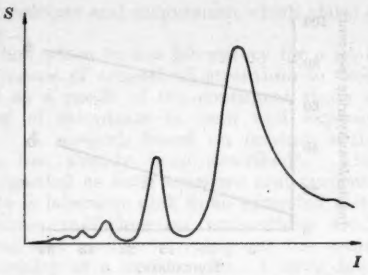


Fig. 1. Intensity of scattered radiation (S) as a function of positive column current (I). (Adapted from A. Dattner, ref. 2)

Microwave radiation of 10-cm. wave-length was coupled to a guide which was terminated by a tapered horn. The horn was arranged so that the E vector of the emergent radiation would be perpendicular to the axis of the plasma column. The plasma was contained in a glass tube of 8 mm. inside diameter and 47 cm. length. The glass tube was located directly in front of the horn at a distance of 30 cm. The discharge 'bulb', which was immersed in an oil bath, contained a mercury pool cathode and a hold anode. The bulb was connected to the lower end of the glass tube.

In order to reduce the amount of radiation scattered by the room walls and other objects, the area around the horn and scattering column was enclosed on the sides by panels of microwave absorbing material. The H component of the field of the scattered radiation was measured as a function of scattering angle using a 7-mm. diameter circular loop placed perpendicular to and 65 cm. distant from the axis of the plasma column. To minimize the amount of direct radiation from the horn to the pickup loop an additional absorbing panel was located between the horn and loop. The position of this panel was adjusted to produce a null output from the detection system at each loop position when the positive column was not ignited. Care was taken to ensure that adjustments of the panel did not measurably affect the intensity of the radiation incident on the column nor shield the loop from the column.

The positive column current was modulated by use of a 50-c./s. sine wave superimposed on a direct current. The amplitudes of the direct and 50-c./s. currents were adjusted so that electron densities corresponding to the five most pronounced resonances were swept through on each cycle.

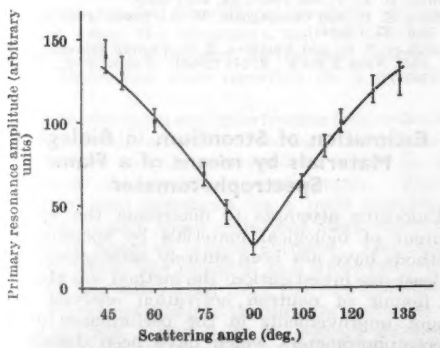


Fig. 2. Amplitude of the primary resonance as a function of the scattering angle

After amplification and detection, the loop output was displayed on the vertical axis of an oscilloscope the horizontal deflexion of which was made proportional to the positive column current. Thus the oscilloscope displayed a plot of the scattered radiation field as a function of the column current at a particular scattering angle. Using the foregoing arrangements, it was possible to determine the amplitude of the three largest resonances for scattering angles, θ, between 45° and 135° with respect to the incident direction.

Fig. 2 shows a plot of the amplitude of the largest or primary resonance as a function of scattering angle. Also shown in Fig. 2 is the curve $25 \sin\theta + 165 \cos\theta$, which is seen to provide a reasonable fit. The addition of the small $\sin\theta$ term to the anticipated $\cos\theta$ dependence of a dipole H field is due to the presence of some electrical pickup by the loop and its cable. There is no evidence of higher multipoles. Thus the primary resonance is dipole. The sizes of the plus–minus bars attached to each point were based on the accuracy with which the null adjustment of the movable absorbing panel could be made and on the noise level of the system.

Table 1. The Ratios of the Secondary (2) and Tertiary (3) to Primary (1) Resonant Amplitudes as a Function of Scattering Angle θ

θ	(2)/(1)	(3)/(1)
45	0·27	0·12
54	0·25	0·13
60	0·23	0·10
75	0·28	0·14
82	0·24	0·09
90	0·23	0·08
97	0·27	0·07
105	0·23	0·10
112	0·27	0·15
120	0·23	0·08
127	0·29	0·12
Average ratio	0·25	0·11
RMS deviation	0·02	0·02

Table 1 gives the ratios of the secondary and tertiary resonant amplitudes to the primary resonant amplitudes as a function of scattering angle. Neither of these ratios have angle dependent trends of sufficient amplitude to indicate any difference in multipolarity among the three resonances. Thus, since the primary resonance is dipole, all three must be dipole. It is perhaps safe to suppose that the other resonances behave similarly, but the noise level of the system prohibited the accurate measurement of the higher modes.

Calculations made by Makinson and Slade[3] appear to predict multiple dipole resonances while other results[4,5] have indicated a single dipole resonance. The present experiment supports the result of Makinson and Slade.

It is a pleasure to acknowledge the extensive assistance given this work by Mr. Adam Dattner and to thank Prof. H. Alfvén for his hospitality, encouragement and the use of laboratory facilities.

Forrest I. Boley*

Department of Electronics,
Royal Institute of Technology,
Stockholm.

* On leave from Wesleyan University, Middletown, Connecticut.

[1] Romell, D., *Nature*, **167**, 243 (1951).
[2] Dattner, A., Ericsson Technics, No. 2, 309 (1957).
[3] Makinson, R. E. B., and Slade, D. M., *Austral. J. Phys.*, **7**, 268 (1954).
[4] Herlofson, N., *Ark. f. Fysik*, **3**, 247 (1951).
[5] Kaiser, T. R., and Closs, R. L., *Phil. Mag.*, **43**, 1 (1952).

Transformation of the α- and β-Forms of Calcium Sulphate Hemihydrate to Insoluble Anhydrite

Although there is no difference between the X-ray diffraction patterns of α- and β-calcium sulphate hemihydrate[1], differences have been reported for density, specific heat and the heat of formation[2]. I have shown that the dehydration of each form to soluble anhydrite proceeds at about the same rate, but that the rate of transformation of soluble to insoluble anhydrite is much greater for the α-form. This is of importance in the examination of gypsum plasters, which may contain one or both of these forms according to the method of manufacture. The difference between the α- and β-forms is demonstrated here by simultaneous differential thermal and thermogravimetric analyses[3], and by determining the rate of the phase transformation at a static temperature.

Samples of α-hemihydrate were prepared by heating a pure precipitated gypsum in concentrated nitric acid[1], in a saturated steam atmosphere[2], and in a concentrated solution of magnesium sulphate[4]. Samples of β-hemihydrate were prepared by calcining the same gypsum in open dishes in an air oven at 150°, 200° and 240° C. The differential thermal curve for α-hemihydrate (Fig. 1, curve *a*) indicates the rapid formation of insoluble anhydrite by a sharp exothermic peak at 255° C. The form of the curve was the same for a large number of samples prepared by the methods outlined above. Although in some instances a reduction in the size of the exothermic peak was noted, the claim by Budnikov and Kosyreva[5], that the differential thermal curve for α-hemihydrate shows no exothermic effect, is not in agreement with my results. The simultaneous thermogravimetric curve showed that the water content of the sample was about 0·1 per cent when the exothermic reaction commenced, and X-ray examination of the sample heated to 290° C. (the end of the exotherm) showed that it contained about 60 per cent insoluble anhydrite. Further conversion of the α-soluble anhydrite to insoluble anhydrite took place slowly between 290° and 550° C., with a loss of about 0·05 per cent of water. The β-hemihydrate behaves differently. The differential thermal curve (Fig. 1, curve *b*) indicates a gradual transformation to insoluble anhydrite commencing at about 300° C. The simultaneous thermogravimetric curve indicates that at 300° C. (the beginning of the exotherm) the water content was reduced to about 0·2 per cent, and X-ray examination of the sample heated to 500° C. (the end of the exotherm) showed that it was almost pure insoluble anhydrite. By comparison, it should be noted that in the decomposition of the α-hemihydrate a small amount of soluble anhydrite still remained at 500° C.

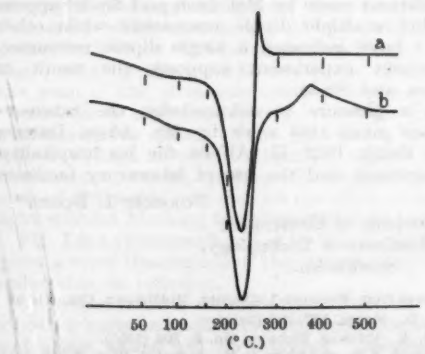


Fig. 1. Differential thermal analysis curves for calcium sulphate hemihydrate. *a*, α-form; *b*, β-form

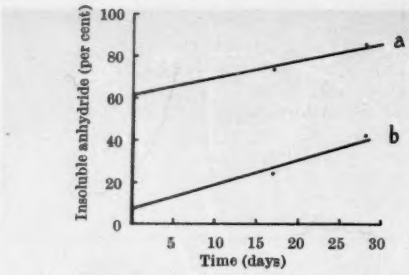


Fig. 2. Rate of formation of insoluble anhydrite from calcium sulphate hemihydrate at 200° C. *a*, α-form; *b*, β-form

In a mixture containing α- and β-hemihydrate the exothermic peak of the α-form is often not visible on the differential thermal curve because it is masked by the endotherm of the β-form, which is not complete until about 300° C.; but the form of the curve is still different and the area of the exothermic peak due to the β-form is significant. It is reduced to about half its normal size in a mixture of equal parts of α- and β-hemihydrates, and can therefore give an indication of the proportion of each form present. The area of each exothermic peak of β-hemihydrate was not appreciably different for samples prepared by heating at 150° C. for 4 hr., at 200° C. for 1 hr., and at 240° C. for 45 min.

The phenomena observed under dynamic heating conditions were also observed at a static temperature of 200° C. Fig. 2 shows that initially the rate of transformation of the α-form to insoluble anhydrite is about eight times greater than that for the β-form, but thereafter the rate decreases to about half that of the β-form.

I wish to thank Dr. W. F. Cole for taking the X-ray photographs.

D. A. Powell

Division of Building Research,
Commonwealth Scientific and
Industrial Research Organization,
Melbourne.
July 14.

[1] Weiser, H. B., and Milligan, W. O., *J. Amer. Chem. Soc.*, **59**, 1456 (1937).
[2] Kelley, K. K., Southard, J. C., and Anderson, C. T., U.S. Bur. Mines Tech. Paper 625 (1941).
[3] Powell, D. A., *J. Sci. Instr.*, **34**, 225 (1957).
[4] Schoch, E. P., and Cunningham, W. A., *Trans. Amer. Inst. Chem. Eng.*, **37**, 1 (1941).
[5] Budnikov, P. P., and Kosyreva, Z. S., *Voprosy Petrograf. i Mineral. Akad. Nauk S.S.S.R.*, **2**, 342 (1953). (*Chem. Abstr.*, **48**, 13314*a* (1954).)

Estimation of Strontium in Biological Materials by means of a Flame Spectrophotometer

Previous attempts to determine the strontium content of biological materials by spectrochemical methods have not been entirely satisfactory, and in at least one investigation[1] the method was abandoned in favour of neutron activation analysis. With recent improvements in the performance of flame spectrophotometers which have been described by various authors[2] methods have been evolved for the

assay of calcium and magnesium which claim a high accuracy.

Need has arisen in this laboratory for a rapid and reliable means of estimating strontium in biological material as a result of the continued study of the turn-over of strontium in man and experimental animals. A method, based on neutron activation analysis, has already been described[3]. Although this is regarded as both sensitive and accurate, the procedure is laborious and, in an extended metabolic study, these radiochemical estimations are time-consuming and involve carrying out the estimation in the vicinity of a nuclear pile. I have devised a rapid and accurate method for the estimation of strontium in which an *S.P.* 900 'Unicam' flame spectrophotometer is used.

(*a*) *Chemical procedure.* Since strontium is only a trace element in biological material, ranging in human plasma from about 0·03 p.p.m. to about 600 p.p.m. in human fæcal ash, some chemical treatment of the material is necessary. (The specimens should contain at least 5 μgm. of strontium.) Urine or plasma was precipitated with excess ammonium oxalate at about neutral *p*H. All solid materials such as bone, tissue or fæces were dry-ashed overnight at about 650° C. The subsequent treatment involved the removal from the ashed material of much of the alkali and phosphate, which otherwise interferes with the spectrochemical estimation of the strontium. All solids were therefore dissolved in 2 *N* hydrochloric acid. (A suitable aliquot was withdrawn at this stage for calcium estimation, which was assayed either by a modified ethylenediaminetetraacetic acid method which is shortly to be described or by means of the flame spectrophotometer.) Strontium and calcium were reprecipitated as oxalates and, after centrifuging and discarding the supernatant, the residues were dissolved in concentrated nitric acid. The concentration of nitric acid was then increased to 85–90 per cent by the addition of fuming acid. After standing in iced water for 10 min., these specimens were centrifuged and the supernatant again discarded. The insoluble nitrates (mainly of calcium and strontium) were then dissolved in water and made up to a concentration of between 1 and 5 p.p.m. in strontium in a suitable volumetric flask (25 or 50 ml., say).

(*b*) *Spectrophotometric assay.* A known aliquot, say 0·1 ml., of a standard strontium solution containing 200 p.p.m. of 'Specpure' strontium was placed in a 10-ml. volumetric flask and this was then filled to the mark with the nitrate extract already prepared. An internal standard is thus obtained and the two solutions were assayed directly on the flame spectrophotometer using the strontium line at 460·7μ, a slit-width of about 0·05 mm. and a gain setting of about 5. Deflexions were recorded on a galvanometer.

In order to eliminate any interference from calcium or other ions on the strontium readings, the galvanometer deflexion was also observed at about 5μ on either side of the strontium wave-length. These settings gave zero deflexions on a pure strontium solution but usually small deflexions were observed in the case of tissue or excreta extracts. The mean deflexion, φ, which was regarded as a white radiation background, was subtracted from the two deflexions θ_1 and θ_2, θ_1 corresponding to the unknown solution of strontium content X and θ_2 to the second solution. Then, if C denotes the known addition of strontium made:

$$\frac{X + C}{X} = \frac{\theta_2 - \varphi}{\theta_1 - \varphi} \text{ or } X = \frac{(\theta_1 - \varphi)C}{\theta_2 - \theta_1}$$

provided the galvanometer readings so corrected are proportional to the concentration of strontium. This condition was verified using a variety of concentrations over the range 0–10 p.p.m. Table 1 shows the comparison between the strontium content for various samples as determined on the spectrophotometer with that determined by neutron activation.

Table 1

Specimen	Strontium content	
	Flame photometer	Neutron activation
Fæcal ash 1	7·28 mgm./day	7·23 mgm./day
Fæcal ash 2	5·28 ,, ,,	5·45 ,, ,,
Fæcal ash 3	5·72 ,, ,,	5·45 ,, ,,
Urine oxalate 1	0·137 ,, ,,	0·153 ,, ,,
Urine oxalate 2	0·187 ,, ,,	0·188 ,, ,,
Cow's milk ash 1	36 p.p.m.	35 p.p.m.
Cow's milk ash 2	49 ,,	37 ,,
Diet ash	74 ,,	80 ,,

This comparison is illustrative of the general agreement which we have obtained between the two methods. It is concluded that the error as deduced from such comparisons as shown in Table 1 and from the reproducibility of individual determinations is between 2 and 3 per cent at strontium concentrations of about 2 p.p.m.

G. E. Harrison

Medical Research Council
Radiobiological Research Unit,
Harwell, Berkshire.

Aug. 18.

[1] Sowden, Eleanor M., and Stitch, S. R., *Biochem. J.*, **67**, 104 (1957).
[2] MacIntyre, I., *Biochem. J.*, **67**, 164 (1957). Exley, D., and Sproat, D., *J. Sci. Instr.*, **35**, 202 (1958). Jury, R. V., Webb, M. S. W., and Webb, R. J., U.K.A.E.A. Report A.E.R.E. C/R2614 (1958).
[3] Harrison, G. E., and Raymond, W., *J. Nuc. Energy*, **1**, 290 (1955).

Observation of Transient Intermediates in Redox Processes by Variable Voltage Oscillo-polarography and Cyclic Voltammetry

The dropping mercury electrode can be used for oscillo-polarography[1] but the oscillo-polarograms obtained during the investigation of organic compounds are often difficult to interpret.

Recent work in this laboratory has led to the development of a 'hanging' mercury drop electrode which has proved of value in the analysis of both inorganic and organic solutions[2,3]. This electrode yields oscillo-polarograms in which a clear and stable image is produced and any desired potential between 0 and − 2 V. can be applied. This feature, in combination with the possibility of using current of any frequency, makes interpretation of the results much easier and also permits the observation of the existence in solution of short-lived intermediates which do not appear in ordinary polarograms.

Some results which confirm this are shown in Fig. 1, the solution employed being a 2×10^{-4} *M* *p*-nitroaniline in 0·1 *N* potassium hydroxide.

The curves shown on the right-hand side of the diagram are (1) the polarograms obtained by use of the dropping electrode, and (2) the hanging drop cyclic voltammetric curves. In the latter it is seen that the first reduction cycle shows only the peak corresponding to the polarographic wave (1).

This corresponds to the following reduction:

$NH_2C_6H_4NO_2 + 6e + 6H^+ \rightarrow NH_2C_6H_4NH_2 + 2H_2O$;

in the first, and subsequent oxidation cycles, however, a minimum at $-0{\cdot}06$ V. is seen and this corresponds to the oxidation:

$$NH_2C_6H_4NH_2 - 2e - 2H^+ \rightleftharpoons NHC_6H_4NH$$

This reaction is reversible and so, in the subsequent reduction cycles, a peak is observed at about $-0{\cdot}10$ V. The above mechanism is supported by our finding that the curves obtained for *p*-phenylenediamine are identical with those discussed here.

The corresponding oscillo-polarograms are shown on the left-hand side of the diagram. The span of the widest curves (7) and (8) corresponds to $-1{\cdot}8$ V., the current being of 50 cycles/sec. Trace (7) corresponds to the cyclic trace (2) on the right-hand side of the diagram. Five 'cuts-in' are seen. Those at about $-0{\cdot}1$ V. correspond to the *p*-phenylenediamine/*p*-di-iminoquinone system while the six electron reduction of *p*-nitroaniline, being irreversible, corresponds to the single cut-in at about $-0{\cdot}80$ V. The pair of cuts-in at $-0{\cdot}50$ V., however, have no parallel in (2) and so must correspond to a new redox system of such low stability as to be detectable only by use of high frequency current.

This system may correspond to the reaction

$$NH_2C_6H_4NO - 2e + 2H^+ \rightleftharpoons NH_2C_6H_4NHOH$$

the nitrosoaniline being derived, possibly, from the incomplete reduction of nitroaniline under oscillopolarographic conditions. The evidence for this supposition is not very strong and further attempts at identification are in progress. Nevertheless, the cuts-in undoubtedly correspond to short-lived species and so it is evident that the method shows promise in such studies.

The traces (3) to (6) illustrate the use of selected applied voltages and the way in which the results can be of value in the interpretation of results. For example, the cuts-in near $-0{\cdot}1$ V. are not observed until the applied voltage reaches $-0{\cdot}6$ V. Thus, these cuts-in are positively identified with the products of the six electron reduction of *p*-nitroaniline. Further evidence is then obtained by comparison with (8), which shows the oscillo-polarogram of *p*-phenylenediamine.

It is evident that variable voltage oscillo-polarography with the hanging drop electrode offers considerable advantages in the interpretation of the sometimes very complicated traces which are often obtained with reducible organic solutions[3], and constitutes a new method of studying the properties of transient intermediates.

W. Kemula
Z. Kublik

Chemical Department,
University of Warsaw.
July 25.

[1] Heyrovsky, J., and Forejt, I., "Oscilloraficka polarografie" (Prague, 1953).
[2] Kemula, W., and Kublik, Z., *Anal. Chim. Acta*, **18**, 104 (1958).
[3] Kemula, W., and Kublik, Z., *Roczniki Chem.* (in the press).

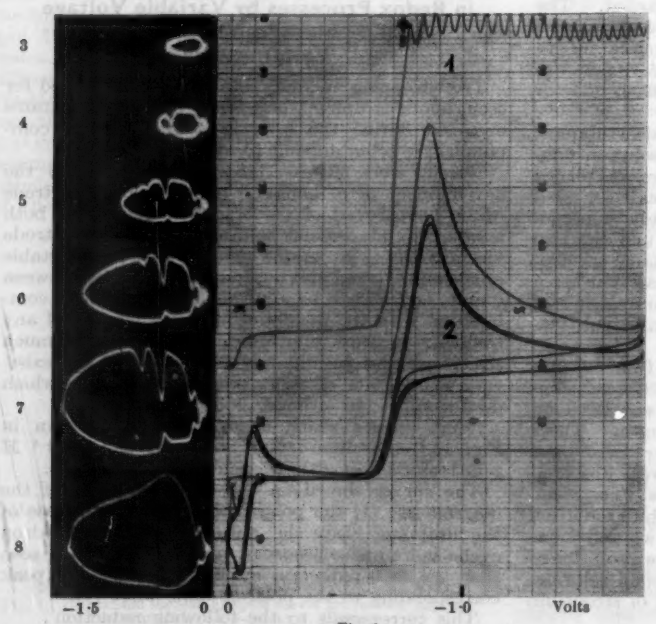


Fig. 1

Standard Potential at 25° C. of the Silver–Silver Chloride Electrode in Formamide

Formamide is a very good solvent for many strong and medium weak acids. In order to determine their dissociation constants accurately by potentiometric measurements it is necessary to know the standard potential of a reference electrode with respect to the normal hydrogen electrode. Until recently, only the value of the cadmium–cadmium chloride electrode has been given in the literature[1]. We have been able to determine the standard potential of the silver–silver chloride electrode by the classical method using the following cell without liquid–liquid junction:

Pt, H_2/HCl($HCONH_2$)/AgCl, Ag

Platinum electrodes were prepared according to Jones[2] and the silver–silver chloride electrodes by the electrolytic method[3,4]. (Both types of electrodes were reproducible within 0·5 mV.)

The potential E of this cell depends on the molality of hydrochloric acid, its mean activity coefficient and the standard electrode potential $E°$. The value of $E°$ may be found by using Hitchcock's method[5], which consists in extrapolating the auxiliary function $E' = E + 2k \log m - 2kAm^{1/2}$ to $m = 0$. Here $k = 2{,}3026\ RT/F$ and $-Am^{1/2}$ is the first Debye–Hückel approximation of the logarithm of the mean activity coefficient. In the case of formamide, with dielectric constant[6] at 25° C. equal to 109·5, $2kA$ is equal to $3{\cdot}65 \times 10^{-3}$.

In all our experiments, the formamide ('Fluka', purum) was purified by vacuum distillation followed by two crystallizations

and a fractional fusion. The physical constants of our final product ($T_f = 2{\cdot}9°$ C.; $n_D^{25} = 1{\cdot}4458$; $\varepsilon^{25} = 109{\cdot}5$) were in excellent agreement with the best values given in the literature[6,7].

Solutions obtained by passing dry gaseous hydrogen chloride into the formamide could not be stored, even in an inert atmosphere. Decomposition of these solutions is probably due to hydrolysis of formamide by very small traces of water, this reaction being catalysed by hydrogen chloride[8]. Every solution used in the determination of $E°$ was therefore prepared directly in its cell immediately before measuring the potential, and the equilibrium values of these potentials, which changed slightly with time, were extrapolated to zero time. Nevertheless, the determination of the standard potential remained difficult as the preparation of the solutions was critical. Owing to the instability of the solutions, accuracy was not better than 1 mV. The molalities used ranged from $2{\cdot}38 \times 10^{-3}$ to $5{\cdot}164 \times 10^{-2}$.

Extrapolation of E' values by the method of least squares gave the following value for the standard potential (Fig. 1):

$$E° = 0{\cdot}204 \pm 0{\cdot}002 \text{ V.}$$

(0·002 is the mean standard deviation of the experimental points with respect to the calculated line.)

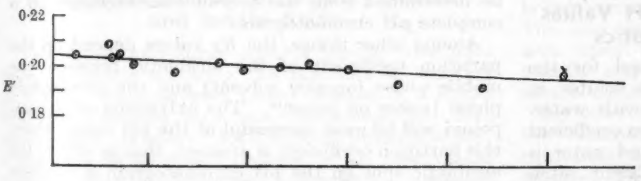


Fig. 1

With the help of this value for $E°$, the dissociation constant of acetic acid could be calculated from the potential of the following cell without liquid–liquid junction:

$$\text{Pt, H}_2/\text{CH}_3\text{COOH, CH}_3\text{COONa, NaCl(HCONH}_2)/\text{AgCl, Ag}$$

Using the method of Harned and collaborators[9], we found $\log K_a = -6{\cdot}81 \pm 0{\cdot}01$ at 25° C., corresponding to a value[10] of the acidity function X equal to 0·578. This is in good agreement with the value predictable from those of other carboxylic acids in formamide and the X value of acetic acid in water[11].

M. Mandel
P. Decroly

Department of Analytical Chemistry,
University of Brussels.

[1] Pavlopoulos, T., and Strelow, H., *Z. phys. Chem.*, N.F., **2**, 89 (1954).
[2] Jones, C., *J. Amer. Chem. Soc.*, **57**, 279 (1935).
[3] Carmody, W., *J. Amer. Chem. Soc.*, **51**, 2901 (1929).
[4] Taylor, J., and Smith, E., *J. Research, Nat. Bur. Stand.*, **22**, 307 (1939).
[5] Hitchcock, D., *J. Amer. Chem. Soc.*, **50**, 2076 (1928).
[6] Leader, G., *J. Amer. Chem. Soc.*, **73**, 856 (1951).
[7] Timmermans, J., "Physico-Chemical Properties of Pure Organic Compounds" (Elsevier, Amsterdam, 1950).
[8] Kreibel, V., and Holst, K., *J. Amer. Chem. Soc.*, **60**, 2976 (1938).
[9] Harned, H., and Owen, B., "The Physical Chemistry of Electrolytic Solutions" (Reinhold, New York).
[10] Mandel, M., *Nature*, **176**, 792 (1955).
[11] Mandel, M., *Anal. Chim. Acta*, **15**, 301 (1956).

Metabolism of Phenolic Compounds by the Broad Bean *Vicia faba*

Phenolic glycosides are common constituents of plant tissues but little is known about the biosynthesis of the glycosidic bond in these compounds. Bourquelot and Hérissey synthesized (*o*-hydroxybenzyl)-β-glucoside by incubating glucose with saligenin in the presence of emulsin[1], but it is unlikely that transglycosylation of this type is an important mechanism for the biosynthesis of glycosides *in vivo*. The formation of aryl glucosides by leaf disks infused with various phenols has been reported by Miwa *et al.*[2] and it appears that glucose-1-phosphate is involved in these reactions.

The stems of young bean shoots were immersed in aqueous solutions containing glucose (0·5 per cent (w/v) and a phenol (0·5 per cent w/v) and after 48 hr. the tissues were extracted with methanol and the extracts examined on paper chromatograms (butan-1-ol/ethanol/water (4 : 1 : 5, organic layer) solvent: ammoniacal silver nitrate[3], diazotized *p*-nitroaniline/sodium hydroxide[4] and periodate–permanganate[5] spray reagents). Some of the products were isolated in small amounts by chromatography on Whatman No. 3 paper and afterwards hydrolysed by an emulsin preparation.

The feeding of quinol and phloroglucinol gave rise to products which co-chromatographed with arbutin (R_F 0·44) and phlorin (R_F 0·35) respectively, and gave the same colour reactions with the diazo reagent as the authentic glycosides. Similarly, the compound obtained by feeding saligenin was indistinguishable from (*o*-hydroxybenzyl)-β-glucoside, and hydrolysis with emulsin yielded saligenin and glucose, this being further evidence for the presence of a β-glycosidic linkage. No evidence for the formation of salicin ((*o*-hydroxymethylphenyl)-β-glucoside) from saligenin could be obtained.

Resorcinol, catechol and hydroxyhydroquinone all gave single products (R_F values 0·51, 0·64 and 0·43 (0·33 on phosphate-buffered paper) respectively), which reacted with the diazo- and periodate-permanganate reagents. Hydrolysis with emulsin in each case produced glucose together with the phenol which had been administered to the shoot.

These results suggest that the mono-β-glucosides of catechol and resorcinol had been produced. The product obtained from hydroxyhydroquinone was probably (3,4-dihydroxyphenyl)-β-glucoside, as it showed a decreased rate of movement when subjected to chromatography on buffered paper, this being evidence for the presence of an *ene-diol* grouping[6]. Small amounts of an electrophoretically and chromatographically identical compound were also formed when young bean shoots were allowed to take up a solution of arbutin (1 per cent w/v). In this case, the arbutin was presumably hydroxylated by polyphenol oxidase.

Glucoside formation could not be demonstrated when phenol was used as a substrate and only traces of possible glucosides were produced using pyrogallol.

The biosynthesis of the glucosides was confined chiefly to the leaves and the activity was completely destroyed by grinding these tissues in the presence of 0·1 *M* phosphate buffer (*p*H 6·0). The synthetic activity of the isolated stems was negligible.

During the preparation of this communication, Hutchinson, Roy and Towers[7] reported glucoside formation by apple-leaf and leaves from a number of other plant species, when fed with phenols in the presence of D-glucose-^{14}C.

This study is being continued to determine the exact mechanism of formation of glucoside in the plant and to examine the biosynthesis and metabolism of the more complex phenolic glycosides containing two or more sugar units.

The interest shown by Prof. E. J. Bourne is very much appreciated.

J. B. Pridham

Department of Chemistry,
Royal Holloway College
(University of London),
Englefield Green, Surrey. July 9.

[1] Bourquelot, E., and Hérissey, H., *C.R. Acad. Sci., Paris*, **156**, 1790 (1913).
[2] Miwa, T., Nakamura, S., and Shibata, A., *Kôso Kagaku Shinpojiuma*, **12**, 48 (1957); *Chem. Abstr.*, **52**, 1314 (1958).
[3] Partridge, S. M., *Biochem. J.*, **42**, 238 (1948).
[4] Bray, H. G., Thorpe, W. V., and White, K., *Biochem. J.*, **46**, 271 (1950).
[5] Lemieux, R. V., and Bauer, H. F., *Anal. Chem.*, **26**, 920 (1950).
[6] Swain, T., *Biochem. J.*, **53**, 200 (1953).
[7] Hutchinson, A., Roy, C., and Towers, G. H. N., *Nature*, **181**, 841 (1958).

A Paper Chromatography Method for the Determination of Suitable *p*H Values for the Extraction of Antibiotics

Extraction methods are usually used for the isolation and purification of antibiotics soluble in organic solvents which are immiscible with water. This method can be applied if the partition coefficient of the antibiotic between the solvent and water is sufficiently large. The partition coefficient often depends on the *p*H value, particularly for acidic or basic compounds.

We have worked out a method for determining suitable conditions for the extraction of antibiotics by a *p*H chromatogram with the help of which we can also investigate the ionic character of the antibiotics. We use *p*H chromatograms when only cultures of the antibiotically active micro-organisms on Petri-dishes are available, that is, even before the crude substances have been isolated.

To prepare *p*H chromatograms we determine the solubility of the compounds by paper chromatography in 10 solvents[1]: distilled water, 3 per cent ammonium chloride (w/v), methanol, acetone, ethyl acetate saturated with water, *n*-butanol saturated with water, ether saturated with water, chloroform, benzene, petrol ether. Agar plugs, diameter 10 mm., cut from the micro-organism colonies, are placed 4 cm. from the lower end of 20 cm. × 1 cm. strips of Whatman No. 1 filter paper. After 15–30 min. the plugs are removed, the strips are dried in air, and chromatographed by the ascending technique using the solvents mentioned. After chromatographing, the strips are dried in air and placed on the surface of agar plates seeded with a suitable test-organism and detected bioautographically in the usual manner. After this, coloured prints on filter paper of the inhibition-zones can be obtained by our method[2].

The *p*H chromatograms are prepared as follows: nine sheets of Whatman No. 1 paper are buffered with McIlvain citrate–phosphate buffers of *p*H 2·2, 3, 4, 5, 6, 7 and 8 and with phosphate buffers of *p*H 9 and 10. After drying in air 20 cm. × 1 cm. strips of the buffered papers are cut, and the starting line and the upper line of the ascending solvents are marked 4 cm. and 19 cm. respectively from the lower end of the papers.

We use one strip of each of the nine buffered papers for the chromatography of every antibiotic. Agar plugs are put on the papers in the same way as was described for the method of selecting a suitable solvent. All nine strips are then chromatographed in the solvent which proved to be the most suitable. Development is stopped when the solvent front reaches the line 15 cm. from the start. After drying the chromatograms in air the bio-autographical detection method is used in the usual way.

The positions of the spots on the *p*H chromatograms are characteristic for every antibiotic. Acidic substances give spots farther from the starting line when chromatographed on papers buffered in the acidic range; the higher the *p*H the smaller the distance of these spots from the starting line. With basic substances the reverse is true. The distances of spots of neutral antibiotics are unaffected by *p*H. In this way the ionic character of an antibiotic can be determined from the overall characteristics of a complete *p*H chromatogram.

Among other things, the R_F values depend on the partition coefficient of the antibiotic between the mobile phase (organic solvent) and the non-mobile phase (water on paper)[3]. The extraction of a compound will be most successful at the *p*H value where this partition coefficient is greatest, that is, where the antibiotic spot on the *p*H chromatogram is farthest from the starting line. On the other hand, re-extraction from the organic solvent to water will proceed best at the *p*H value where the antibiotic spot remains on the starting line, that is, the *p*H at which it is most insoluble in the solvent used.

We have tested our method on a number of commercially available antibiotics using the solvents which are used in their industrial production. The results in all cases indicated extraction procedures absolutely identical with those applied in industry. A more detailed paper on this method will be published later[4].

As an example, a *p*H chromatogram of benzylpenicillin, developed by using amylacetate, is shown in Fig. 1. The appearance of the *p*H chromatogram confirms the acidic character of this compound and

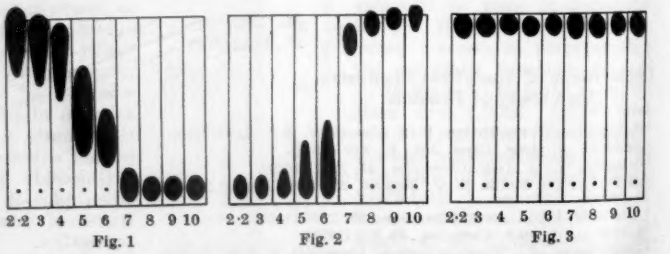


Fig. 1 Fig. 2 Fig. 3

Fig. 1. *p*H chromatogram of benzylpenicillin developed in amylacetate. Test organism, *Bacillus subtilis SDPC* 1 : 220

Fig. 2. *p*H chromatogram of erythromycin developed in amylacetate. Test organism, *Bacillus subtilis ATCC* 6633

Fig. 3. *p*H chromatogram of chloramphenicol developed in ethylacetate. Test organism, *Bacillus subtilis ATCC* 6633

indicates that the extraction of penicillin from water to amylacetate will be best at about *p*H 2. Similarly, the re-extraction from amylacetate to water will be best at *p*H 8. These results agree with known extraction and purification methods[5].

Figs. 2 and 3 show *p*H chromatograms for a basic antibiotic, erythromycin, and a neutral one, chloramphenicol. For erythromycin *p*H 9 and *p*H 4 are most suitable for extraction and re-extraction respectively[5]. The extraction of chloramphenicol is unaffected by *p*H [5,6].

This method has proved to be very helpful in research on new antibiotics.

VLADIMÍR BETINA
Department of Technical Microbiology
and Biochemistry,
Slovak Polytechnical University, Bratislava.

[1] Ševčík, V., Podojil, M., and Vrtišková, A., *Csl. mikrobiol.*, **2**, 175 (1957).
[2] Betina, V., and Pilátová, L., *Csl. mikrobiol.*, **3**, 202 (1958).
[3] Block, J. R., Durrum, E. L., and Zweig, G., "A Manual of Paper Chromatography and Paper Electrophoresis" (New York, 1955).
[4] Betina, V., *Chemické zvesti* (in the press).
[5] Underkofler, L. A., and Hickey, R. J., "Industrial Fermentations," 2 (New York, 1954).
[6] Herold, M., Vondráček, M., Nečásek, J., and Doskočil, J., "Antibiotika" (Prague, 1957).

Exogenous Sources of Urinary Phenol and Indole Acids

THE phenol and indole acids of human urine have been examined following the ingestion of bananas or coffee. Bananas were tested because of a report that they contain serotonin, norepinephrine, dopamine and possibly 3,4-dihydroxyphenylalanine[1]. Coffee was investigated because of an observation that vanillic acid is excreted following the ingestion of caffeic acid, but not coffee[2], and a suggestion that the excretion of vanillic acid may be related to norepinephrine metabolism and stress[3].

Two healthy adults were placed on a high-protein diet from which materials of plant origin were excluded in order to minimize the excretion of exogenous phenol and indole acids. Control urines were collected for 6 hr. on the second and third days. One subject then ingested 4 bananas (480 gm.) and the other 1,000 ml. of coffee percolated from 64 gm. of commercial, ground, roasted coffee beans. Urines were collected for the following 0–7 and 7–15 hr. periods. Extraction and two-dimensional paper chromatography were carried out as described previously[4,5]. The changes observed in the pattern of urinary phenol and indole acids are summarized in Table 1. The amounts of 5-hydroxyindoleacetic, 3-methoxy-4-hydroxy-D-mandelic and 3-methoxy-4-hydroxyphenylacetic acids were determined on paper chromatograms by visual comparison of spots obtained from appropriate aliquots of banana urine extracts and the authentic compounds.

It has been pointed out that measurement of urinary 5-hydroxyindoleacetic acid after ingestion of bananas could result in a diagnosis of carcinoid tumour[6]. The data in Table 1 show that excretion of this serotonin metabolite increased sharply in the first 7 hr. after ingestion but soon returned to normal. The diagnosis thus might vary with the time and duration of the urine collection after ingestion of bananas. The possibility of a diagnosis of pheochromocytoma also has been suggested[1]. The excretion of 3-methoxy-4-hydroxy-D-mandelic acid increased after ingestion of bananas, but the normal range[7] was not exceeded; if the output of this substance is used as an index of norepinephrine metabolism[7], an erroneous diagnosis is therefore unlikely. The excretion of 3-methoxy-4-hydroxyphenylacetic acid, which is a metabolite of 3,4-dihydroxyphenylalanine and dopamine, also remained within the normal range[8] after ingestion of bananas.

No other 5-hydroxyindole acids were detected in the banana urine extracts, even when volumes equivalent to 4 mgm. of creatinine were chromatographed. The occurrence in bananas of at least two 5′-hydroxyindoles besides serotonin has been mentioned[1]. The excretion of indoles No. 14 and 15 increased after banana ingestion; these compounds frequently are present in urines from institutionalized patients[5], but also have been observed in some normal urines.

The same *m*-hydroxyphenyl and 3-methoxy-4-hydroxyphenyl acids which were excreted after coffee (Table 1) also were detected in urine extracts

Table 1. PAPER CHROMATOGRAPHY OF URINARY INDOLE AND PHENOL ACIDS AFTER INGESTION OF BANANA OR COFFEE*

Compound No.†	Urinary acid	Banana			Coffee		
		Control	0–7 hr.	7–15 hr.	Control	0–7 hr.	7–15 hr.
Indole							
1	5-Hydroxyindoleacetic	3·0 mgm./day	13·2 mgm.	0·79 mgm.	++	++	++
2	Indoleacetic	+++	++++++	+++	+++	+++	+++
10	N-Acetyltryptophan	0	0	0	0	+++	+
14	Unidentified	+	++	++	0	0	0
15	Unidentified	+	++	++	0	0	0
Phenol							
2	*m*-Hydroxyphenylhydracrylic	0	0	+	+	+	++++
3	*m*-Hydroxyhippuric	+	+	+	+	+	+++
7	3-Methoxy-4-hydroxyphenylacetic	3·3 mgm./day	1·00 mgm.	1·54 mgm.	+	+	+
8	Unidentified	0	0	0	0	++	+
10	3-Methoxy-4-hydroxy-D-mandelic	2·3 mgm./day	0·40 mgm.	0·19 mgm.	+	+	+
11	*m*-Hydroxyphenylacetic	0	0	++	0	0	0
12	*p*-Hydroxybenzoic	+	+++	+++	+	++	++
16	Feruloylglycine	0	+	0	0	++	++++
17	Vanilloylglycine	0	+	0	0	+	++
18‡	3-Methoxy-4-hydroxyphenylhydracrylic	0	0	0	0	++	++++
19	Vanillic	0	0	0	0	+++	+
20	Dihydroferulic	0	0	0	0	+	+++
21	*m*-Hydroxyphenylpropionic	0	0	0	0	0	+
31‡	Ferulic	0	0	0	0	+	++
34	Unidentified	0	0	0	0	0	+
37	Unidentified	0	0	0	0	+++	+

* One + represents a spot diameter of ¼ in. The total amount of 5-hydroxyindoleacetic, 3-methoxy-4-hydroxy-D-mandelic, and 3-methoxy-4-hydroxyphenylacetic acids in each banana urine extract is corrected for endogenous excretion, which was determined from control urines.
† Numbers refer to chromatographic maps of urinary phenols (ref. 4) and indoles (ref. 5).
‡ These compounds give blue spots with *p*-dimethylaminobenzaldehyde after several hours, and are probably the ones designated as indoles No. 11 and 28 in a previous report (ref. 5).

after ingestion of caffeic or ferulic acids. The results differ in some respects from those of Booth *et al.*[8], who first reported the unusual formation of these groups of metabolites from caffeic and ferulic acids. These workers noted that only *m*-hydroxyhippuric acid and *m*-coumaric glucuronide, and none of the other caffeic acid metabolites, were excreted after ingestion of 'instant' or caffeine-free 'soluble' coffee. Their observation of fewer metabolites may reflect a loss or change of some components during manufacture of 'soluble' coffee. The hydracrylic acids (phenols No. 2 and 18) were not recorded as metabolites by Booth *et al.*[8]. These compounds are converted easily to the corresponding acrylic acids, however, and could have been destroyed under the different conditions of processing.

Vanillic acid and the other related exogenous 3-methoxy-4-hydroxyphenyl acids have been observed in many human urines[4]. Metabolic precursors are also present in plant materials other than coffee, such as fruits and vegetables[2], and natural vanilla flavour is added to some commercial foods. A suggestion that, on an unrestricted diet, the excretion of vanillic acid may be related to norepinephrine metabolism and stress[3] therefore seems unjustified.

This research was generously supported by a grant from the Ford Foundation.

K. N. F. Shaw
J. Trevarthen
Gates and Crellin Laboratories of Chemistry,
California Institute of Technology,
Pasadena, California.

[1] Waalkes, T. P., Sjoerdsma, A., Creveling, C. R., Weissbach, H., and Udenfriend, S., *Science*, **127**, 648 (1958).
[2] Booth, A. N., Emerson, O. H., Jones, F. T., and DeEds, F., *J. Biol. Chem.*, **220**, 51 (1957).
[3] Smith, P., and Bennett, A. M. H., *Nature*, **181**, 709 (1958).
[4] Armstrong, M. D., Shaw, K. N. F., and Wall, P. E., *J. Biol. Chem.*, **218**, 293 (1956).
[5] Armstrong, M. D., Shaw, K. N. F., Gortatowski, M. G., and Singer, H., *J. Biol. Chem.*, **232**, 17 (1958).
[6] Anderson, J. A., Ziegler, M. R., and Doeden, D., *Science*, **127**, 236 (1958).
[7] Armstrong, M. D., McMillan, A., and Shaw, K. N. F., *Biochim. Biophys. Acta*, **25**, 422 (1957).
[8] Shaw, K. N. F., McMillan, A., and Armstrong, M. D., *J. Biol. Chem.*, **226**, 255 (1957).

Rennin Coagulation of Enzymatically Dephosphorylated Casein

Many of the properties of milk depend on the existence of large aggregates of casein[1], the 'molecular' weights of which are in the region of 10^8. When calcium-binding substances such as citrate or oxalate are added, these aggregates are rapidly reduced in size[2] to a 'molecular' weight of about 10^6, the specific value being highly dependent on *p*H, temperature and ionic strength[3]. The mechanism whereby calcium effects the formation of the aggregates is unknown, but it has often been assumed that the first step is combination with the phosphate groups of casein, and it has been suggested[4] that the calcium ions might cross-link adjacent casein molecules. In recent years it has been found that various phosphatases will catalyse the removal of phosphate groups from casein under mild conditions of *p*H and temperature[5,6]. Thus enzymatic dephosphorylation should be a useful tool in studying the role of casein-bound phosphate in the reactions of milk dependent on calcium. The clotting of milk by the enzyme rennin is dependent on calcium[1], and is a reaction which lends itself to precise study.

Several phosphatases were investigated; potato phosphatase was chosen for its apparent freedom from proteinases and for its ability to act on casein as it exists in milk, as well as on solutions of isolated casein. This phosphatase was purified by a slight modification of Kornberg's method (Horecker, B. L., private communication). The final preparation had a specific activity of $1{\cdot}7 \times 10^5$ units/mgm. protein and showed a 1,200-fold increase (on a protein basis) over the original extract. (One unit releases 1 μgm. phosphorus/hr. from *p*-nitrophenyl phosphate in the following conditions: $0{\cdot}02\ M$ substrate; $0{\cdot}08\ M$ acetate buffer (acetic acid/sodium acetate), *p*H 5·0; $0{\cdot}004\ M$ magnesium chloride; incubated at 37° C. A solution containing 1 gm. bovine albumin per dl. was used as a diluent for the enzyme.)

Preliminary studies showed that the rennin coagulation-time of milk could be increased markedly by treatment with potato phosphatase. In some experiments coagulation did not occur even after 100 times the normal amount of rennin had been added. It was then decided to make a quantitative study of this phenomenon, and for this purpose it was thought advisable to work with isolated casein. In order to obtain reproducible results it was necessary to follow a carefully controlled procedure both in the preparation of dephosphorylated casein and in the measurement of rennin coagulation-times. Depending on the degree of dephosphorylation desired, varying amounts of phosphatase (200–1,000 units per ml. of casein solution) were added to a 5 gm./dl. solution of bovine casein (prepared by the method of Van Slyke and Baker[7]), which was then incubated at 37° C. (Solution of the casein was achieved by the slow addition of $0{\cdot}2\ N$ sodium hydroxide to a suspension of the casein in distilled water; the *p*H did not exceed 8 in the process, and the final *p*H was 6·8.) After 0–5 hr., an aliquot was removed for the assay of inorganic phosphate, and the remainder was diluted with 8 volumes of cold (0–5° C.) distilled water and precipitated by the slow addition of $1\ M$ acetic acid. The precipitate was separated by filtration through a sintered glass funnel, then washed 2–5 times with cold distilled water, twice with cold (−13° C.) 95 per cent ethanol and twice with cold (−13° C.) ether. Finally, it was ground in a mortar and dried *in vacuo* over calcium chloride. The product was free from phosphatase activity. For studying their coagulation by rennin, the samples of dephosphorylated casein were dissolved as before to give a solution of 0·3 gm./dl. and then twice dialysed for at least 2·5 hr. at 0–5° C. against 18 volumes of a *p*H 6·8 buffer[8] containing $3{\cdot}66 \times 10^{-3}\ M$ cacodylic acid, $2{\cdot}94 \times 10^{-3}\ M$ triethanolamine, and sufficient calcium chloride to give the desired concentration of calcium. After dialysis the solutions were diluted with buffer to give a final concentration of protein of 0·24 gm./dl. Coagulation times[9] were measured on duplicate samples after 10–15 min. equilibration at 30° C. Crystalline rennin from calves was used (Ernstrom, C. A., unpublished work).

Fig. 1 shows the results obtained with one set of samples of dephosphorylated casein. As the percentage dephosphorylation is increased, the time required for coagulation by rennin first increases and then passes through a maximum. The same behaviour is observed at all three levels of calcium, but the effect is more striking at the lower levels. No coagulation was observed in $0{\cdot}011\ M$ calcium chloride for the samples with intermediate degrees of dephosphorylation. Another set of samples of dephos-

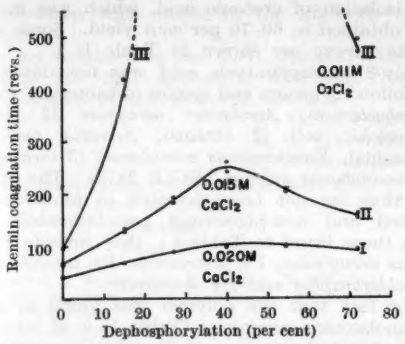


Fig. 1. The relation between percentage dephosphorylation of of bovine casein and the time required for clotting by rennin, at three concentrations of calcium. 10 ml. of casein solution was brought to the temperature of the bath (30° C.), and 1 ml. of rennin ($5{\cdot}6 \times 10^{-4}$ mgm. of rennin nitrogen) was added. Samples were rotated at 16·2 r.p.m. The concentrations of calcium given are those of the buffers against which the samples were dialysed

phorylated casein showed the same behaviour as that in Fig. 1 but gave slightly different values for the coagulation-times. The fact that the curves pass through a maximum suggests that more than one effect of dephosphorylation is present; it is likely that the increase in isoelectric point on dephosphorylation tends to produce shorter coagulation times.

We wish to acknowledge the assistance, at various times, of D. B. Gammack, J. J. Kelley and Dr. M. Lapidus, and also financial support from the Carnation Co. and from the Continental Can Co. This communication is published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

Robert Y. H. Hsu
Laurens Anderson
R. L. Baldwin
C. A. Ernstrom
A. M. Swanson

Departments of Biochemistry and
Dairy and Food Industries,
College of Agriculture,
University of Wisconsin,
Madison 6, Wisconsin.
June 19.

[1] Reviewed by Pyne, G. T., *Dairy Sci. Abstr.*, **17**, 532 (1955).
[2] Philpot, F. J., and Philpot, J. St. L., *Proc. Roy. Soc.* B., **127**, 21 (1939).
[3] von Hippel, P. H., and Waugh, D. F., *J. Amer. Chem. Soc.*, **77**, 4311 (1955).
[4] Reported by McFarlane, A. S., *Nature*, **142**, 1023 (1938).
[5] Reviewed by Perlmann, Gertrude E., "Adv. in Protein Chem.", **10**, 1 (1955).
[6] Sundararajan, T. A., and Sarma, P. S., *Biochem. J.*, **65**, 261 (1957).
[7] Van Slyke, L. L., and Baker, J. C., *J. Biol. Chem.*, **35**, 127 (1918).
[8] Gorini, L., and Lanzavecchia, G., *Biochim. Biophys. Acta*, **14**, 407 (1954).
[9] Sommer, H. H., and Matsen, Helene, *J. Dairy Sci.*, **18**, 741 (1935).

'Solubilization' of a Cytochrome *b* Component from *Micrococcus lysodeikticus*

In an earlier communication[1] we reported some preliminary observations on a cytochrome *b* component of *Micrococcus lysodeikticus*, strain N.C.T.C. 2665. The cytochrome was separated to some extent from the cytochromes *a* and *c* which were also present, but it remained attached to particles which were thrown down by centrifugation at 20,000*g*.

In further experiments, we have found that the cytochrome can be 'solubilized' by treatment of the particulate preparation with a 1 per cent solution of 'Cetrimide' (B.P.). Centrifugation at 20,000*g* at this stage separated a small deposit which contained some cytochrome *c*. Further purification of the cytochrome *b* component was achieved by fractional precipitation from the supernatant by addition of ammonium sulphate. Between 20–40 per cent (w/v) ammonium sulphate, the small quantity of residual cytochrome *c* was precipitated. The cytochrome *b* component came down as a red, gelatinous precipitate at ammonium sulphate concentrations of 40–60 per cent (w/v). This material was apparently soluble in 0·04 *M* phosphate buffer at *p*H 7. The solution obtained was dialysed for 24 hr. against running tap-water, and during this time precipitation of a yellow pigment occurred, the absorption spectrum of which suggested that it might be a carotenoid. The maxima in petroleum ether, 415 mμ, 435 mμ and 465 mμ, corresponded to the wave-lengths recently reported[2,3]. The precipitate was filtered off and the filtrate was allowed to stand in the cold. More of the yellow pigment separated over a period of 48 hr., but no further precipitation occurred during the subsequent 72 hr.

The remaining solution was examined spectrophotometrically, after reduction by addition of solid sodium dithionite, and peaks corresponding to a cytochrome of the *b*-group were found. The absorption maxima of this component were at 558 mμ, a small peak at approximately 525 mμ and a Soret peak at 529–530 mμ; there was no evidence of residual cytochrome *c*. It appears, therefore, that the component should be regarded as a cytochrome b_1. The maximum at 558 mμ differs slightly from that found earlier with cruder preparations in glycerol, which showed a 'flat' peak extending from 560 mμ to 563 mμ. These observations have been repeated, and the slight difference has been confirmed. Whether it is due to the presence of an extra component in the crude preparations or to a slight shift due to different optical or physico-chemical conditions is not yet certain. Because of the residual light-scattering with the cruder materials, the readings were necessarily less precise than with the clear solutions which were later available.

The solutions obtained by the method described still contained some yellow carotenoid pigment which could not be extracted with petroleum ether. The pigment was, however, extractable with chloroform or butanol, but the cytochrome b_1 was damaged by this treatment.

These observations suggest that at least some of the carotenoid is conjugated with a protein, and while it is possible that this might be the protein moiety of cytochrome b_1 itself, we have no direct evidence for this.

We wish to thank the Medical Research Council for a grant, and Dr. D. W. Henderson, of the Microbiological Research Department, Porton, who kindly arranged the bulk culture of *Micrococcus lysodeikticus*.

F. L. Jackson
Valerie D. Lawton

Department of Bacteriology,
King's College Hospital Medical School,
Denmark Hill,
London, S.E.5.
July 10.

[1] Jackson, F. L., and Lawton, Valerie, D., *Nature*, **181**, 1539 (1958).
[2] Litwack, G., and Carlucci, A. F., *Nature*, **181**, 904 (1958).
[3] Gilby, A. R., and Few, A. V., *Nature*, **182**, 55 (1958).

Occurrence of Poly-β-Hydroxybutyric Acid in Aerobic Gram-Negative Bacteria

During an examination of the metabolic products of an unidentified purple-pigmented pseudomonad, a fraction, soluble in chloroform and insoluble in ether, was found to comprise 30 per cent of the dry cell weight. The material was isolated as a colourless solid, m.p. 168° C., which gave on alkaline hydrolysis a 70 per cent yield of crotonic acid. (Identified by m.p., mixed m.p. and preparation of the anilide and *p*-bromphenacyl ester.) The crotonic acid could also be obtained in 60–70 per cent yield by destructive distillation of the original solid. It was thought likely that the solid was a β-hydroxybutyric acid polymeric ester and this was confirmed by infra-red spectroscopy (ref. 1 and Farmer, V. C., personal communication) and by analysis (found, per cent; C, 55·1; H, 7·1. Calculated for $(C_4H_6O_2)_n$: C, 55·8; H, 6·8). Similar polymers have been described from *Bacillus megaterium*[2], *Azotobacter chroococcum*[3] and *Micrococcus halodenitrificans*[4].

The unidentified pseudomonad (*HCC*, *B* 175)[5], which was typical of a group isolated from Trinidad soils, was stained for the presence of 'fat' by the method of Burdon[6], using an alcoholic solution of sudan black *B* (G. T. Gurr, Ltd., London). The cells were found to be intensely sudanophilic, as was the isolated polymer. A survey was then made for the presence of sudanophilic material in a wide range of bacteria grown on an agar medium containing glucose or glycerol as major source of carbon. All those which stained strongly and a number which did not stain were further examined. Shake flask cultures or cultures from a 2-litre aerated fermenter were centrifuged, and the dried cells extracted with hot chloroform. The chloroform extract was filtered into 3 volumes of ether, and the formation of a flocculent white precipitate was taken as being indicative of the presence of poly-β-hydroxybutyric acid. No organism lacking in sudanophilic substance gave this reaction, although some containing sudanophilic substance did not contain the polymer and presumably contained conventional lipids. The presence of poly-β-hydroxybutyric acid was confirmed in representative cultures by destructive distillation of the solid and isolation of crotonic acid, which was in every case obtained in 60–70 per cent yield. Some results of the survey are shown in Table 1.

Poly-β-hydroxybutyric acid was not detected in the following genera and species of bacteria: *Proteus*, *Flavobacterium*, *Aerobacter aerogenes* (2 strains), *Escherichia coli* (2 strains), *Serratia marcescens* (5 strains), *Xanthomonas vasculorum* (3 strains) and *Protaminobacter rubrum* (*NCIB* 2879). The polymer has thus far not been detected in numerous pigmented and non-pigmented pseudomonads, other than those listed in Table 1; they include *Pseudomonas aeruginosa*, *Ps. fluorescens*, *Ps. aureofaciens*[8,9], *Ps. chlororaphis* and *Ps. lemonnieri*[10].

The fact that the polymer was found in all the chromobacteria examined (Table 1) is of interest in view of the observation of Sneath[11] in a taxonomic study of *Chromobacterium*, that the mesophilic chromobacteria, especially, contain considerable deposits of 'fat'.

Root nodules of several leguminous plants were examined in addition to cultures of *Rhizobium*. Acetone-dried nodules from *Mimosa pudica* were found to contain 6 per cent of poly-β-hydroxybutyric acid. This substance was also detected in nodules of a *Pueraria* sp., but apparently in smaller amount. Crushed nodules from both plants were stained with sudan black *B* and the bacteroids found to contain much sudanophilic material.

Our survey has been confined mainly to aerobic Gram-negative bacteria. Poly-β-hydroxybutyric acid has not, however, so far been detected in *Streptomyces* or *Lactobacillus*, in the anaerobe, *Clostridium acetobutylicum*, or in several yeast-like fungi.

We have isolated from sea water a salt-tolerant Gram-negative rod capable of forming in artificial culture large quantities of poly-β-hydroxybutyric acid. We have not yet examined cultures of *Spirillum* for the presence of poly-β-hydroxybutyric acid, though the evidence of Lewis[12] on the cytology of this genus leads us to suspect that some spiral bacteria contain large deposits of this substance.

The cultural conditions affecting yields of polymer and the enzymic mechanism of synthesis are being studied further and will be reported elsewhere. It is obvious that there are many cases in the literature where deposits of poly-β-hydroxybutyric acid have been confused with true lipids or even as in the case

Table 1

Culture	Source and strain No.*	Culture medium (carbohydrate and organic nitrogen sources only)	Poly-β-hydroxybutyric polymer (percentage of cell dry weight)	Melting point (° C.)
Chromobacterium violaceum	*NCIB* 8182	2 per cent glucose/peptone (68 hr.)	37	164
Chromobacterium sp.	*HCC* Strain 1	2 per cent glucose/peptone (72 hr.)	33	167
Rhizobium sp.	From *Indigofera* sp. *HCC B*141	2 per cent mannitol/asparagine/yeast extract (69 hr.)	19	164
Rhizobium sp.	From *Pueraria* sp. *HCC B*142	2 per cent mannitol/asparagine/yeast extract (86 hr.)	57	176
Azotobacter vinelandii	University of Wisconsin. Strain *O*†	2 per cent mannitol/asparagine/yeast extract (69 hr.)	24	178
Pseudomonas solanacearum	Wilted tomato plant. Strain *F*2/2	2 per cent glycerol/peptone (36 hr.)	6·5	172
Pseudomonas solanacearum	Diseased banana plant. Strain *RE*21	2 per cent glucose/peptone (68 hr.)	30	172
Pseudomonas antimycetica (ref. 7)	*HCC B*44	2 per cent glycerol/peptone (89 hr.)	8	167
Pseudomonas sp.	Trinidad soil *HCC B*175	2 per cent glycerol/peptone (48 hr.)	31	168

Melting points quoted above are uncorrected.

* *HCC*, Hankey Culture Collection, Colonial Microbiological Research Institute, Port of Spain, Trinidad; *NCIB*, National Collection of Industrial Bacteria, Teddington, Middlesex.

† Supplied by Prof. P. W. Wilson, University of Wisconsin.

In addition to the bacteria listed above, poly-β-hydroxybutyric acid polymer has been identified in nineteen cultures of *Chromobacterium* and four of *Rhizobium*, two cultures of *Azotobacter agilis*, five cultures of *Pseudomonas solanacearum* isolated from diseased bananas in Trinidad, and in fifteen Pseudomonads related to *Pseudomonas* sp. *HCC B*175 isolated from Trinidad soils.

of *Rhizobium* with stages in the life-cycle of the organism.

We are indebted to Dr. V. C. Farmer, Macaulay Institute for Soil Research, Aberdeen, for the determination of the infra-red spectra.

W. G. C. FORSYTH
A. C. HAYWARD
J. B. ROBERTS
Colonial Microbiological Research Institute,
Port of Spain,
Trinidad.
June 24.

[1] Blackwood, A. C., and Epp, A., *J. Bact.*, **74**, 266 (1957).
[2] Lemoigne, M., Delaporte, B., and Croson, M., *Ann. Inst. Pasteur*, **70**, 224 (1944).
[3] Lemoigne, M., and Girard, H., *C.R. Acad. Sci., Paris*, **217**, 557 (1943).
[4] Smithies, W. R., Gibbons, N. E., and Bayley, S. T., *Can. J. Microbiol.*, **1**, 605 (1955).
[5] Morris, M. B., and Roberts, J. B. (unpublished work).
[6] Burdon, K. L., *J. Bact.*, **52**, 665 (1946).
[7] Thaysen, A. C., and Thaysen, I., VIth Int. Congr. Microbiol., Rome, Proc., **1**, 321 (1953).
[8] Kluyver, A. J., *J. Bact.*, **72**, 406 (1956).
[9] Haynes, W. C., Stodola, F. H., Locke, J. M., Pridham, T. G., Conway, H. F., Sohns, V. E., and Jackson, R. W., *J. Bact.*, **72**, 412 (1956).
[10] Hugo, W. B., and Turner, M., *J. Bact.*, **73**, 154 (1957).
[11] Sneath, P. H. A., *J. Gen. Microbiol.*, **15**, 70 (1956).
[12] Lewis, I. M., *J. Bact.*, **40**, 271 (1940).

Separation of Anthocyanin and Leuco-anthocyanin in Flowers of *Camellia japonica*

BATE-SMITH[1] observed that a methanol extract of white flowers of *Camellia japonica* turned red after heating with dilute hydrochloric acid. This suggests that leuco-anthocyanin is present in the white flowers. By the same procedure I found that red flowers of several horticultural varieties of *C. japonica* contained leuco-anthocyanin yielding cyanidin, as well as a glycoside of cyanidin.

A methanol-hydrochloric acid extract of red flowers of one of the varieties, Shikainami, was paper-chromatographed with the solvents butanol/concentrated hydrochloric acid/water (5 : 1 : 4, v/v) and 80 per cent formic acid/concentrated hydrochloric acid/water (5 : 1 : 4, v/v)[2]. As controls, chrysanthemin and cyanin were chromatographed on the same paper. R_F values were estimated as 0·24, 0·27 and 0·09, respectively, with the former solvent; and 0·53, 0·58 and 0·75 with the latter. The hydrolysate of the extract and cyanidin were then co-chromatographed using the second solvent. R_F values of the samples were identical, that is 0·20. No other spots of anthocyanin or anthocyanidin could be detected on the chromatograms.

The separation of leuco-anthocyanin from anthocyanin was carried out as follows. The red flowers (20 gm. fresh weight) were macerated with 150 ml. methanol and filtered. The filtrate was concentrated *in vacuo* at 30° C. until 15 ml. of a dark purplish-red syrup remained. The syrup was passed through a resin column (1·3 × 12 cm.) packed with 'Amberlite' *IRC*–120 (H form). The anthocyanin was found to be adsorbed quantitatively on the resin and the filtrate from the column was slightly orange. The filtrate was neutralized with 0·1 *N* sodium hydroxide and saturated with sodium chloride. Then it was exhaustively shaken with 30 ml. portions of ethyl acetate until the aqueous layer remained orange-brown after heating with propanol-hydrochloric acid[3]. The combined colourless ethyl acetate extracts were dried with sodium sulphate and evaporated *in vacuo* until a slightly pink-coloured residue remained. The residue was dissolved in 7 per cent hydrochloric acid and heated in a water-bath until a dark red colour appeared. The red matter was transferred by partition to a small amount of amyl alcohol, and the alcoholic layer was paper-chromatographed, in parallel with cyanidin, with formic acid/hydrochloric acid as solvent. The R_F value of the former was 0·19 (accompanied by brownish trail), and that of the latter (cyanidin) 0·20. This indicates that the leuco-pigment is sugar-free and should be regarded as leuco-cyanidin[4]. Extract of red flowers of the other variety, Somekawa, showed almost the same results.

Thus, it appears that flowers of some varieties of *C. japonica* contain leuco-anthocyanin, irrespective of their flower colours.

TORU ENDO
National Institute of Genetics,
Misima, Sizuoka-Ken,
Japan.
June 20.

[1] Bate-Smith, E. C., *J. Exp. Bot.*, **4**, 1 (1953).
[2] Endo, T., *Jap. J. Bot.*, **14**, 187 (1954).
[3] Pigman, W., *et al.*, *Tappi*, **36**, 4 (1953).
[4] Robinson, G. M., and Robinson, R., *J. Chem. Soc.*, 744 (1935).

Incorporation of Orotic Acid-^{14}C and Lysine-^{14}C in Regenerating Single Nerve Cells

EXPERIMENTS on the cytoscale and also, by inference, on the tissue and macroscale have demonstrated that proteins, lipids and ribonucleic acid in the central nervous system are in a dynamic state[1–3]. The production and metabolism of these substances are apparently promoted by increased functional demands.

The process of peripheral nerve regeneration can be repeated several times during the life-cycle of the organism. The production of proteins, lipids and ribonucleic acid per nerve cell has been studied by quantitative cytochemical methods during regeneration in hypoglossal cells[4]; the latent out-growth and maturation period could clearly be distinguished cytochemically. The results reported here demonstrate the incorporation of lysine-^{14}C into the nerve-cell proteins, and orotic acid-^{14}C into the ribonucleic acid of nerve cells during regeneration.

Nerve cells belonging to the hypoglossal nucleus in rabbits were used. The nerve was crushed with cooled forceps and 0·01 mc. L-lysine-^{14}C or orotic acid-^{14}C was injected intracisternally. The rabbits treated with lysine were killed 30 min. after the injection, and the others 24 hr. after the administration of orotic acid. The nerve cells were sampled by dissecting by hand under a preparation microscope. Fig. 1 shows some fresh hypoglossal cells, dissected out and photographed in the phase-contrast microscope. Five to ten cells were used for each measurement. The cells were transferred rapidly from the fresh tissue to 0·25 *M* sucrose, freed from surrounding glial cells, placed on a holder and treated with 1 *N* cold perchloric acid for 30 sec., and carefully washed and dried. The hypoglossal control cells then weigh on average 2,200 μμgm., that is 2,000 μμgm. protein and about 10 per cent ribonucleic acid. The activity was measured with a special Geiger microtube with a window thickness of 1·5 mgm./cm.^{2}, provided

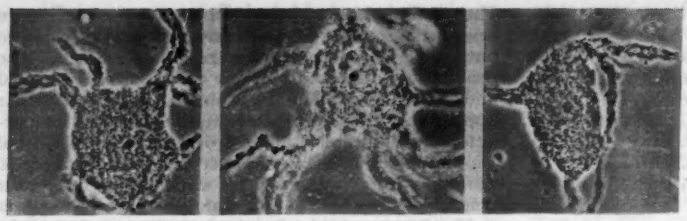


Fig. 1. Fresh hypoglossal cells from the rabbit photographed in the phase-contrast microscope in 0·25 *M* sucrose. Note the synaptic knobs as small granules on the surface of the cells

with an anti-coincidence shield. The background activity of the tube at the measuring was only 1 count/min. The results obtained are related below to those found in the quantitative study on the same type of material[4].

During the 'latent period' a small part of the nerve close to the site of the crush degenerates. The amount of protein per nerve cell was found to decrease by 30–40 per cent during this stage. The cell volume began to increase, and morphologically chromatolytic areas could be found within the cell. The incorporation of lysine-^{14}C into regenerating cells exceeded that into control cells by 70 per cent (Table 1).

Table 1. Uptake of Lysine-^{14}C by Regenerating Isolated Nerve Cells

Days after operation	Activity ratio operated cell/control cell
2	1·7
9	3·2
21	1·5
207	1·4

Lajtha *et al.*[3] and Furst *et al.*[5] have pointed out that lysine is especially useful in the study of brain proteins. In experiments of short duration the incorporation into rapidly metabolized proteins is primarily measured. The presence of free lysine can be expected to influence the result slightly, but it is important to note that this factor does not affect the conclusion that the activity found in our experiments is a measure of the metabolic activity of the cell proteins.

The protein decrease per cell and the increased incorporation of lysine into the cell proteins show that the protein loss during the latent period is not compensated by intracellular production, in spite of increased protein synthesis.

During the next stage, 3–12 days after the crush, the thin axons rapidly grow out to re-establish contact with the effector organ. This 'out-growth period' is characterized by a 100 per cent increase in the proteins per cell. The incorporation of lysine into the regenerating cells was found to increase by 200 per cent. During the same period no significant change occurred in the amount of ribonucleic acid per cell; this remained at 200 μμgm. But a most significant alteration occurs in the aggregation of the cytoplasmic ribonucleic acid which becomes finely dispersed, with the typical appearance of chromatolysis. During the first day after the crush no difference in the orotic acid-^{14}C incorporation was found in control and regenerating nerve cells. On the eighth day, however, when chromatolysis was pronounced, the incorporation increased by 100 per cent in the regenerating cells.

Thus, the nerve cell showing typical retrograde changes during regeneration is characterized by an intracellular concentration decrease and a considerable increase in the rate of protein metabolism. Furthermore, the orotic acid incorporation and the change in aggregation of ribonucleic acid indicate that the ribonucleic acid of the chromatolytic nerve cell is transformed from a less active to a more active form.

During the 'maturation period' the size of the axon increases. The incorporation of lysine indicates that the increase in protein metabolism per nerve cell will continue during this stage. Fischer *et al.*[6] found that regenerating hypoglossal cells 14 days after a nerve crush incorporated more methionine-^{35}S per unit area than did the control cells.

A detailed account of the experiments will be published elsewhere. The studies have been supported by grants from the Rockefeller Foundation and from the Swedish Medical Research Council.

S.-O. Brattgård
H. Hydén
J. Sjöstrand

Department of Histology,
University of Göteborg.
June 3.

[1] Hydén, H., "Biochem. of the Developing Nervous System", 358 edit. by Waelsch H., 358 (Academic Press, New York, 1955).
[2] Lajtha, A., Furst, S., and Waelsch, H., *Experientia*, **13**, 168 (1957).
[3] Geiger, A., Yamasaki, S., and Lyons, R., *Amer. J. Physiol.*, **184**, 239 (1956).
[4] Brattgård, S.-O., Edström, J.-E., and Hydén, H., *J. Neurochem.*, **1**, 316 (1957).
[5] Furst, S., Lajtha, A., and Waelsch, H., *J. Neurochem.*, **2**, 216 (1958).
[6] Fischer, J., Lodin, Z., and Kolousek, J., *Nature*, **181**, 341 (1958).

Influence of Linoleic and Linolenic Acids on Symptoms of Vitamin E Deficiency in Chicks

Exudative diathesis and encephalomalacia, two of the outstanding symptoms of vitamin E deficiency in chicks, are much promoted by dietary polyenoic fatty acids[1,2]. It has recently been shown[3] that, in certain circumstances, the former can occur even when the diet is free from polyenoic fatty acids; further, it can be prevented by a trace of 'Selenite'[4,5]. To our knowledge, encephalomalacia has never been observed with diets not containing polyenoic fatty acids, and this symptom is not prevented by 'Selenite'[6].

The differences in the etiology of exudative diathesis and encephalomalacia clearly call for further study. We have therefore studied the influence of two different polyenoic fatty acids, namely, linoleic and linolenic, on the two symptoms.

The composition of the vitamin E – free basal diet used is shown in Table 1. Analyses of the yeast and gelatine (petroleum ether extraction from acidified alkali-hydrolysate and subsequent alkali-isomerization) showed that the amount of polyenoic fatty acids in this diet was less than 0·016 per cent.

The ethyl esters of linoleic and linolenic acids were added daily at a level of 1·5 per cent to the basal diet. (In our Department it has been found that Fleischmann yeast, Type 50*B*, has a powerful antioxidant effect, so that poly-unsaturated fatty acids incor-

Table 1. Composition of Basal Diet

	(gm./100 gm.)
Fleischmann yeast, type 50*B*	40
Gelatine	3
Salt mixture (ref. 7)	5·17
Vitamin mixture (ref. 7)	0·10
Choline chloride	0·20
Sucrose	51·53

Plus 0·001 gm. dicalcium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric acid ester ('Synkavit', Roche) per 100 gm. diet. Vitamins A and D_3 were given in aqueous solution (ref. 7), 0·1 ml. twice a week each animal, corresponding to 250 I.U. vitamin A and 20 I.U. vitamin D_3 a day.

porated in a diet of the composition mentioned are only slightly autoxidized when left on open trays for a period of 24 hr. at 26° C. This is contrary to what happens when casein is the source of protein in the diet. The ethyl esters of linoleic and linolenic acids were obtained from the Hormel Institute, Austin, Minn. The linolenate, which had been prepared via the hexabromide, contained some trans-fatty acids as shown by infra-red spectrophotometry.)

Chicks which had received a commercial chicken mash low in vitamin E during the first two weeks after hatching[7] and thereafter were reared on the experimental diets showed the symptoms listed in Table 2 within 28 days.

It is evident that exudative diathesis was produced by both the two polyenoic fatty acids examined, but encephalomalacia only occurred with linoleic acid.

About one-third of the chicks with exudates in the linoleate and linolenate groups had peroxides (values ranging from 3·8 to 49·3) in the affected fat tissue. No peroxides could be detected in the fat tissue of the chicks which had no exudates.

The fact that the incidence and severity of exudative diathesis was less in the linoleate group than in the linolenate group may be due to the fact that, in the linoleate group, 11 out of the 12 chicks died from encephalomalacia before the end of the experimental period. In the linolenate group, however, 9 out of 12 chicks lived through the entire experimental period.

The observation that linoleic acid produces encephalomalacia in the absence of vitamin E is in accordance with the results of earlier experiments in which peanut oil treated with 'Filtrol' was used as addition to an otherwise fat-free and vitamin E – free diet[8].

The present experiments suggest that a suitable diet for producing encephalomalacia may be one containing linoleic acid and a trace of 'Selenite' in order to prevent simultaneous occurrence of exudative diathesis.

Since dietary linoleic acid is, to a large extent, converted into tetraenoic (arachidonic) acid, it is possible that linoleate exerts its encephalomalacia producing effect via arachidonate. Earlier studies[9] have demonstrated that the fraction of hog liver fatty acids which remains in solution in acetone at −76° C. has a powerful effect in provoking encephalomalacia.

Table 2. Experimental Feeding Period: 28 Days

Addition to basal diet	No. of chicks in group at beginning of experiment	No. of chicks in group alive at end of experiment	No. of chicks with exudative diathesis	No. of chicks with encephalomalacia
None	12	12	0	0
1·5 per cent ethyl linoleate	12	1	3*	11
1·5 per cent ethyl linolenate	12	9	7†	0

* None of the exudates were so severe that they affected the general behaviour of the chicks.
† Four of these chicks had very severe exudates.

This fraction is known to contain arachidonic acid. Addition of cholesterol to a diet producing encephalomalacia decreases the incidence of encephalomalacia and favours the development of exudative diathesis[1]. This may be related to the observation that, when added to a diet containing linoleic acid, cholesterol decreases the deposition of tetraenoic acid in the liver[9]. However, further studies are required to investigate more fully the part played by tetraenoic and other polyenoic fatty acids as well as their isomers in vitamin E-deficiency symptoms.

The dietary level of linoleic acid used in the present experiments corresponds approximately to the amount of this acid in a diet containing 60 per cent torula yeast '3*N*' without added fat[6]. Nevertheless, such a torula yeast diet produces only a few cases of encephalomalacia and a very high incidence of exudative diathesis. This is suggestive of the occurrence in torula yeast of principles counteracting encephalomalacia and favouring exudative diathesis. The existence of factors provoking exudate, in addition to the particularly effective polyenoic fatty acids has already been proved, since we have found that silver acetate added at a level of 20 p.p.m. silver ions to the basal diets mentioned and certain other basal diets has a pro-exudative effect.

H. Dam
G. Kofoed Nielsen
I. Prange
E. Søndergaard
Department of Biochemistry and Nutrition,
Polytechnic Institute, and
The Danish Fat Research Institute,
Copenhagen.
June 12.

[1] Dam, H., *J. Nutr.*, **27**, 193 (1944).
[2] Dam, H., *J. Nutr.*, **28**, 297 (1944).
[3] Dam, H., Kofoed Nielsen, G., Prange, I., and Søndergaard, E., *Experientia*, **14** (in the press).
[4] Patterson, E. L., Milstrey, R., and Stokstad, E. L. R., *Proc. Soc. Exp. Biol. Med.*, **95**, 617 (1957).
[5] Schwarz, K., Bieri, J. G., Briggs, G. M., and Scott, M. L., *Proc. Soc. Exp. Biol. Med.*, 95, 621 (1957).
[6] Dam, H., Kofoed Nielsen, G., Prange, I., and Søndergaard, E., *Experientia*, **13**, 493 (1957).
[7] Dam, H., Hartmann, S., Jacobsen, J. E., and Søndergaard, E., *Acta Physiol. Scand.*, **41**, 149 (1957).
[8] Søndergaard, E., Prange, I., and Dam, H., Vitamina E. Attl del terzo Congresso Internazionale, Venezia, 1955, p. 329.
[9] Dam, H., and Kofoed Nielsen, G., *Acta Physiol. Scand.*, **37**, 359 (1956).

Inability of Epinephrine to protect Rhode Island Chicks against the Lethal Action of X-Rays

Stearner *et al.* have observed that young White Leghorn chicks[1] are protected against radiation mortality by epinephrine in solution in oil. I have repeated these experiments with Rhode Island chicks, which are more radio-resistant than White Leghorns, reproducing Stearner's conditions so far as the number and time of injections and the amounts of epinephrine injected are concerned.

Twenty-one day old Rhode Island chicks were irradiated with 2,000-r. X-rays, administered at a dosage-rate of 100r./min. An oily solution of epinephrine (Parke Davis) was injected into the pectoral muscles 5 min. before and 5 min. after irradiation, 2·5 or 5 mgm./kgm. being injected each time.

I have also used epinephrine, ephedrine and privine (2-naphthyl-(1′)-methyl-imidazoline nitrate) in aqueous solution.

Table 1. Effect of Intramuscular Injections of Epinephrine in Peanut Oil on Initial and Delayed Mortality of X-irradiated Rhode Island Chicks
(Total dose, 2,000 r., dosage-rate 100 r./min.)

Dose of epinephrine before and after X-rays (mgm./kgm.)	No. of animals	Total mortality at 30 days (per cent)	Initial mortality at 0–2 days (per cent)	Delayed mortality at 3–30 days (per cent)
Controls	25	84	64	20
2·5	20	85*	60*	25*
5	25	80*	72*	8*

* Variations not statistically significant

After 30 days, the mortality among the irradiated chicks treated with epinephrine in oil was the same as that of the control chicks irradiated with 2,000 r. (Table 1). Initial mortality (0–2 days) was higher in animals injected with 5 mgm. of epinephrine in oil per kgm. body-weight than in irradiated controls.

Although I used the same technique as Stearner and his collaborators for injecting epinephrine obtained from the same source, I have been unable to reproduce with Rhode Island chicks the results obtained by these workers with White Leghorns[2]. My techniques differed from theirs in that I used a higher dosage-rate of irradiation (100 r./min.) and a higher total dose, and in the age and the race of the chicks employed. However, in my experiments a total dose of 2,000 r. administered to my control chicks gave a mortality after 30 days approximately equal to that found by the American authors[1] after 15 days in White Leghorns irradiated with 1,000 r. Not only did I find that no protection was afforded by epinephrine dissolved in oil, but mortality was accelerated among irradiated chicks when 5 mgm./kgm. was administered before and after irradiation.

Some assays have shown that epinephrine and ephedrine in aqueous solution have no protective action[3]. Privine, which is also a vasoconstrictor in the case of mammals, is very toxic (apathy, paralysis, dyspnea) and totally ineffective in the case of X-ray sickness in chicks.

M-L. Beaumariage
Department of General Pathology,
University of Liège.
June 30.

[1] Stearner, S. P., *Amer. J. Roentgenol. and Rad. Therapy*, **65**, 265 (1951).
[2] Stearner, S. P., Christian, E. J., and Brues, A. M., *Amer. J. Physiol.*, **176**, 455 (1954).
[3] Beaumariage, M-L., *Internat. J. Appl. Rad. Isotopes* (in the press).

Effect of Analogues of Glutathione on the Feeding Reaction of Hydra

Loomis[1] has shown that the 'feeding reaction' which *Hydra littoralis* exhibit after they have captured living prey is controlled chemically: the feeding reaction is elicited by glutathione released from the prey. In the absence of prey, glutathione induces writhing of the tentacles and, in higher concentrations, glutathione causes the hydra to retract the tentacles. These effects were found to be more marked when the hydra had previously been starved for several days. Loomis found that the effect was quite specific and that even the closely related aspartathione was inactive. Two analogues of glutathione (ophthalmic acid and norophthalmic acid) have recently been isolated from calf lens[2–4]; they differ structurally from glutathione in that the SH group is replaced by a CH_2 group in ophthalmic acid, and an H atom in norophthalmic acid. We have now found that these analogues of glutathione also elicit the feeding reactions in *Hydra littoralis*.

The experimental conditions were the same as those used by Loomis. The hydra were kept in a modified tap water medium[5], and were starved for five days before each experiment; during starvation the medium was renewed daily. The results are shown in Table 1; the activity is classified as strongly marked (++), clearly discernible (+), doubtful (±) or absent (0). Ophthalmic acid is seen to be more active, and norophthalmic acid less active, than glutathione, which itself is active at a concentration of only 10^{-6} *M*. Since the analogues do not contain an SH group, it follows that the SH group of glutathione is here unnecessary; thus for the first time a specific biological effect of glutathione is found to be unrelated to the SH group, but strongly dependent upon the molecular size and shape.

Table 1. Activities of Glutathione, Ophthalmic Acid and Norophthalmic Acid in Eliciting the Feeding Reaction of *Hydra littoralis*

Concentration (*M*)	Activity*		
	Glutathione	Ophthalmic acid	Norophthalmic acid
10^{-3}	++	++	+
10^{-4}	+	++	±
10^{-5}	+	++	0
10^{-6}	0	+	
10^{-7}	0	0	

* See text for description of categories.

Two analogues of glutathione which do not occur naturally have also been tested; these are inactive themselves, but inhibit the elicitation of the feeding response by glutathione or ophthalmic acid. The results for the adduct obtained from glutathione and N-ethylmaleimide are shown in Table 2; the activities are classified as described above. The other synthetic inhibitor is the sulphonic acid, γ-L-glutamyl-L-sulphoalanylglycine, and this gave the same pattern of results. Furthermore, similar results were obtained when these synthetic inhibitors were tested against the feeding reaction elicited by ophthalmic acid; norophthalmic acid, itself less active, was more readily inhibited. Thus, of the analogues of glutathione, the two naturally occurring ones are active, but the two synthetic ones are inactive and inhibitory. In the glyoxalase reaction, however, where glutathione is a coenzyme and its SH group enters into the reaction, both the naturally occurring and synthetic analogues are inhibitors[6,7].

Ophthalmic acid was not detected in the *Artemia* on which the hydra fed, so that there is no evidence that the feeding reaction elicited by ophthalmic acid occurs under ordinary conditions.

We are greatly indebted to Dr. W. F. Loomis for supplying us with *Hydra littoralis*; we are also

Table 2. Activity of Glutathione in the Presence of an Inhibitor (the Adduct of Glutathione and N-ethylmaleimide) in Eliciting the Feeding Reaction of *Hydra littoralis*

Concentration of inhibitor (*M*)	Concentration of glutathione (*M*)	Ratio of concentration of inhibitor to concentration of glutathione	Activity*
10^{-3}	10^{-3}	1	++
10^{-3}	10^{-4}	10	+
10^{-3}	10^{-5}	100	0
10^{-3}	10^{-3}	1	+
10^{-3}	10^{-3}	10	±
10^{-3}	10^{-4}	100	0

* See text for description of categories.

grateful to the Distillers' Company, Ltd., for a gift of glutathione, and to Miss Stewart for care of the hydra; one of us (E. E. C.) is in receipt of a Thornton bursary.

E. E. CLIFFE
S. G. WALEY

Nuffield Laboratory of Ophthalmology,
University of Oxford.
July 14.

[1] Loomis, W. F., *Ann. N.Y. Acad. Sci.*, **62**, 209 (1955).
[2] Waley, S. G., *Biochem. J.*, **64**, 715 (1956).
[3] Waley, S. G., *Biochem. J.*, **67**, 172 (1957).
[4] Waley, S. G., *Biochem. J.*, **68**, 189 (1958).
[5] Loomis, W. F., and Lenhoff, H. M., *J. Exp. Zool.*, **132**, 555 (1956).
[6] Kermack, W. O., and Matheson, N. A., *Biochem. J.*, **65**, 48 (1957).
[7] Waley, S. G., Biochem. Soc. Symposium (in the press).

Adrenal Activity in Subjects with Complete Transverse Lesions of the Spinal Cord

HUMAN subjects with complete transverse lesions of the upper thoracic and cervical spinal cords show some features of hypoactivity of the adrenal medulla and of the adrenal cortex: the concentration of adrenaline in the peripheral plasma[1] and the excretion of 17-ketogenic steroids in the urine[2] are both significantly lower than normal.

Since eosinophilia is commonly found in conditions of adrenal insufficiency it was of some interest to measure the number of eosinophils in the blood of patients with complete transverse lesions of the upper thoracic and cervical cord, that is above the fifth thoracic vertebra (T.5), and to compare them with the values found in patients with cord lesions below T.5. This latter group of patients showed no features of adrenal hypoactivity. Eosinophil counts were carried out by the method of Pilot[3].

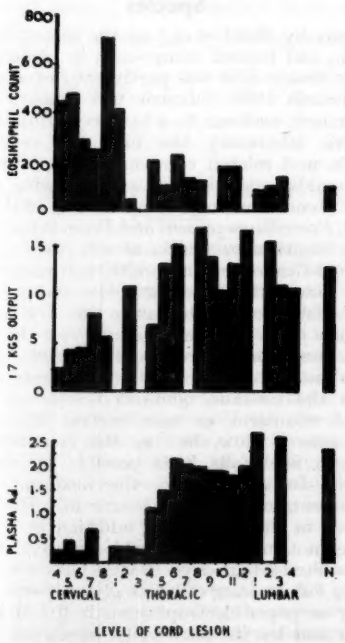


Fig. 1. Mean values of eosinophil count in cells per mm.³ (top), 17-ketogenic steroid excretion in mgm. per 24 hr. (middle), and plasma adrenaline in μgm./l. (bottom). The columns marked 'N' show the mean results of groups of normal control subjects

Thirteen out of twenty-one patients with complete spinal cord lesions above T.5 showed some degree of eosinophilia with counts above 300 per mm.³; twenty-three patients with lesions complete below T.5 were all normal.

The accompanying figure shows the mean values for plasma adrenaline, 17-ketogenic steroid excretion and circulating eosinophils in patients with complete transverse lesions of the spinal cord at levels from the fourth cervical to the fourth lumbar vertebra.

Patients with high cord lesions tend to have high eosinophil counts, low values for the plasma adrenaline and low excretions of 17-ketogenic steroids; patients with lesions of the lower thoracic and lumbar cords show normal values.

Long[4] has developed the idea that adrenaline, which is assumed to be released during stress, stimulates the secretion of adrenocorticotrophic hormone. He found that the adrenal cholesterol and ascorbic acid fell under these circumstances and also after adrenaline infusion.

Our investigations were carried out on fit subjects with spinal cord lesions of long standing who were not under stress. It is interesting that the association between the activity of the adrenal cortex, as measured by the eosinophil count and the excretion of 17-ketogenic steroids, and the output of adrenaline still appears to hold even in circumstances of subnormal adrenaline output.

R. ROBINSON

National Spinal Injuries Centre,
Stoke Mandeville Hospital,
Aylesbury.

A. F. MUNRO

Dept. of Physiology,
King's College,
University of London.

[1] Munro, A. F., and Robinson, R., *J. Physiol.*, **141**, 4P (1958).
[2] Robinson, R., *Nature*, **181**, 484 (1958).
[3] Pilot, *Brit. Med. J.*, 4741 (1951).
[4] Long, C. N. H., in "Symposium on Stress" (U.S. Army Medical Service Graduate School, Washington, 1951).

Acetylcholine in Mammalian Neuromuscular Transmission

AFTER reviewing experimental results described in the literature, Acheson[1] came to the conclusion in 1948 that the amount of acetylcholine liberated by a single impulse at a presynaptic nerve terminal in muscle and sympathetic ganglia is probably of the order of $1{\cdot}5 \times 10^{-16}$ gm. (10^{-18} moles). He pointed out the large discrepancy between this quantity and the relatively large amounts (3×10^{-14} moles) hitherto found necessary for excitation when applied directly to the muscle. This discrepancy has been much reduced in recent years by the electrophoretic application of acetylcholine from micropipettes[2,7], and spikes have been produced in frog muscle fibres by this method with as little as $5{\cdot}5 \times 10^{-16}$ moles[7]. However, there still remained a substantial difference, and most of the potentials obtained by this method have not been strictly comparable with end-plate potentials elicited by nerve stimulation since they have a much slower time-course. For example, the quickest potential published by del Castillo and Katz[3] rose to its maximum in 13 msec. These authors concluded that much faster potentials, and even greater sensitivity to acetylcholine, would be expected in the case of nervous release of acetylcholine, where the diffusion distance is less than 1μ; in their

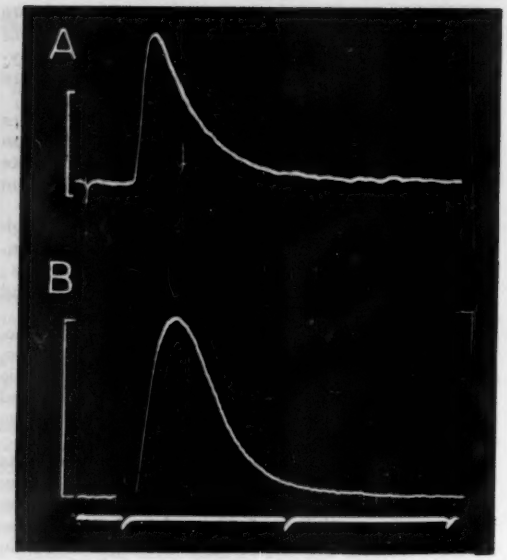


Fig. 1. *A*, End-plate potential evoked by stimulation of phrenic nerve at 2 c./s. The muscle was paralysed by 15·0 m*M* magnesium. *B*, Acetylcholine potential evoked in another muscle by a 1 msec. pulse of current ($3·5 \times 10^{-6}$ amp.) at a rate of 1 c./s. In both cases, muscles were rat diaphragms, at 24–25° C.; the potentials were recorded with intracellular electrodes. Voltage scale: 5 mV. in *A* and 10 mV. in *B*. Common time scale: 10 msec.

experiments, the distance of the acetylcholine pipette from the end-plate was estimated to be 10–20μ (in a later paper[4], they mention potentials with a rise time of 2–5 msec.).

By the use of a similar technique, we have found that acetylcholine potentials could be produced in the rat diaphragm with amounts as small as $1·5 \times 10^{-17}$ moles (assuming a transport number of 0·3 for ACh+). The experiments were performed *in vitro*, at room temperature (24–25° C.), with an intracellular recording electrode (filled with 3 *M* potassium chloride) in the end-plate region of a muscle fibre; jets of acetylcholine were delivered from an external micro-pipette filled with 2–4 *M* acetylcholine (as chloride, Roche Products) by square outward current pulses lasting 0·1 to 1·0 msec. The lowest values were obtained with 0·1 msec. pulses. No allowance was made for the appreciable loss of current to be expected with such short pulses in view of the relatively high pipette resistance and capacitance to earth.

The amplitude and time course of the fastest acetylcholine potentials were extraordinarily sensitive to the slightest disturbance. The fastest potentials had a time course comparable with that of end-plate potentials evoked by nerve stimulation. Fig. 1 shows on the same time scale the potential produced with a 1 msec. pulse of acetylcholine ($3·5 \times 10^{-11}$ coulombs or 10^{-16} moles) and a typical end-plate potential in another diaphragm paralysed by 15·0 m*M* magnesium (the voltage scales differ by about 15 per cent). The rise time to the maximum of the acetylcholine potential is approximately twice as long as the corresponding time for the end-plate potential (1·3 msec.). A time lag of about 3 msec. is consistent with a diffusion distance of about 4μ. The peak of the acetylcholine potential is somewhat more rounded than that of the end-plate potential, but its rate of fall is fairly similar, so that the total durations of the two potentials differ by relatively little.

It seems that with the present technique an even closer approximation to nervous transmission is likely to be limited by the width of the end plate (19–28μ in the rat diaphragm[2]). The rounded peak of the acetylcholine potential might well be caused by saturation of the receptors closest to the pipette.

Although some discrepancy may therefore be expected to remain, the value computed somewhat indirectly by Acheson (10^{-18} moles) is quite likely to be too small by a factor of at least 10, as suggested by Acheson himself, since most experimental errors would tend to reduce it. As a further source of error, presynaptic failure of nerve conduction may well occur during repetitive stimulation[5]; it is particularly likely under conditions of reduced oxygen supply[6] such as would be expected during the perfusion of muscle with Locke solution as in the experiments reviewed by Acheson.

K. Krnjević
R. Miledi*

Physiology Department,
Australian National University,
Canberra.

* Rockefeller Foundation Fellow.

[1] Acheson, G. H., *Fed. Proc.*, **7**, 447 (1948).
[2] Cole, W. V., *J. Comp. Neurol.*, **108**, 445 (1957).
[3] del Castillo, J., and Katz, B., *J. Physiol.*, **128**, 157 (1955).
[4] del Castillo, J., and Katz, B., *J. Physiol.*, **132**, 630 (1956).
[5] Krnjević, K., and Miledi, R., *J. Physiol.*, **140**, 440 (1958).
[6] Krnjević, K., and Miledi, R., *J. Physiol.*, (in the press).
[7] Nastuk, W. L., *Fed. Proc.*, **12**, 102 (1953).

Metabolism of Coumarin and Related Compounds in Cultures of *Penicillium* Species

Studies by Mead *et al.*[1] on the metabolic fate of coumarin and related compounds in rabbits showed that *o*-coumaric acid was partly converted to 4-hydroxycoumarin while coumarin was converted neither to *o*-coumaric acid nor to 4-hydroxycoumarin.

In this laboratory the biological oxidation of coumarin and related compounds has been studied using moulds isolated from soil, following the technique of Henderson and Farmer[2]. Two of the moulds isolated, *Penicillium jenseni* and *Penicillium nigricans*, are able to utilize coumarin as sole carbon source in a modified Czapek medium, although rate of growth is very slow. During growth the concentration of coumarin falls steadily, but at no time is it possible to isolate any other coumarin derivatives. In a similar medium containing sucrose and coumarin, growth is vigorous, and a little umbelliferone is detectable.

When the medium contains *o*-coumaric acid in place of coumarin as sole carbon source, again growth rate is slow, but as the concentration of *o*-coumaric acid falls it is possible by chloroform extraction to isolate from the medium relatively large amounts of 4-hydroxycoumarin. The presence of sucrose in the medium in addition to *o*-coumaric acid does not interfere with this hydroxylation.

The isolated 4-hydroxycoumarin has been identified by its R_F values using ethylene glycol systems[3], by its mobility on paper electrophoresis in 0·2 *M* borate at *p*H 8·5, and by its ultra-violet spectrum in cyclohexane.

Paper chromatography and electrophoresis of the chloroform-soluble matter in the medium also revealed

a compound with R_F values, and mobilities identical with those of dicoumarol (3 : 3-methylene-bis-4-hydroxycoumarin), but so far in too small a quantity to allow of purification for spectrophotometric comparison with pure dicoumarol. Growth of either species of *Penicillium* in a medium containing 4-hydroxycoumarin and sucrose also provided evidence for the presence of dicoumarol. In every case uninoculated control solutions showed no traces of the conversions described above.

It appears that *o*-coumaric acid, and not coumarin, may be converted to dicoumarol in cultures of *Penicillium* species isolated from soil. This is significant in view of the investigations[4] which led to the identification of dicoumarol as the hæmorrhagic agent in spoiled sweet clover hay. An early report by Smith and Brink[5] stated that alfalfa hay, containing no coumarin derivatives, developed hæmorrhagic properties when mixed with coumarin and allowed to ferment, and it has been generally supposed that coumarin is the precursor of the dicoumarol formed in fermented sweet clover hay. However, Stahmann *et al.*[4] commented on the small fraction of the total coumarin present which was apparently converted to dicoumarol. *o*-Coumaric acid is present in much lower concentrations in general, and a substantial conversion of this substance to dicoumarol by contamination of the hay with soil fungi could lead to concentrations of dicoumarol of the order of those found.

Fuller details of this work will be published in due course.

David M. Bellis

Department of Pharmacy,
The University,
Manchester, 13.
July 18.

[1] Mead, J. A. R., Smith, J. N., and Williams, R. T., *Biochem. J.*, **68**, 67 (1958).
[2] Henderson, M. E. K., and Farmer, V. C., *J. Gen. Microbiol.*, **12**, 37 (1955).
[3] Bellis, D. M., and Rigby, G. J., *Nature*, **178**, 415 (1956).
[4] Stahmann, M. A., Huebner, C. F., and Link, K. P., *J. Biol. Chem.*, **138**, 513 (1941).
[5] Smith, W. K., and Brink, R. A., *J. Agric. Res.*, **57**, 145 (1938).

Abnormally Low Ultimate *p*H in Pig Muscle

The *p*H attained when the process of post-mortem glycolysis has ceased—the ultimate *p*H[1]—is influenced by the type of muscle considered, the species in which it occurs and the physiological condition of the animal from which it is derived. In the longissimus dorsi (l. dorsi) muscles of horses[2], oxen[3] and pigs[4], the glycogen reserves of which have not been depleted by such stresses as pre-slaughter exercise, starvation or excitement, an ultimate *p*H of about 5·5 is characteristic. At this *p*H the enzymes which effect glycolysis are assumed to be inactivated, and residual glycogen may be found. Stress, by depleting glycogen reserves at or before death, is therefore normally associated with values of ultimate *p*H above 5·5.

Some observations on pig muscles having an ultimate *p*H considerably lower than 5·5 are now briefly reported. Superficially these muscles are characterized by their loose texture, their exudative condition and, generally but not always, by the fact that they are almost white whereas normal pork is pink to light red in colour.

Fig. 1. Longitudinal section of pig semimembranosus muscle, *p*H 4·94. Note absence of cross-striations in left-hand fibre and twisting and corrugation of the other two. Fixed in formalin : celloidin embedded. Stained : hæmatoxylin, van Gieson. (× 200)

This 'white muscle' condition has aspects in common with "muskeldegeneration" in Danish[5] pigs and with "la myopathie exsudative dépigmentaire du porc" in France[6,7], but there are also features which appear to distinguish it from these and other distrophic disorders of muscle.

In the present work an ultimate *p*H of 5·1 or below was frequently found, the lowest value being 4·78 in l. dorsi. The latter and the semimembranosus appear to be particularly susceptible to the condition. Susceptibility also varies within a single muscle. White and pink locations in the semimembranosus only 1 cm. apart have had ultimate *p*H values of 4·94 and 5·56 respectively. In l. dorsi muscles having low *p*H there is a lower content of acid-soluble phosphorus (about 1·8 mgm./gm. compared with 2·0 mgm./gm. in l. dorsi of normal pigs) and a lower buffering power ($5·66 \times 10^{-5}$ equivalent/*p*H/gm. compared with about $5·9 \times 10^{-5}$ normally).

Histologically there are several unusual features, the degree of abnormality being related to the ultimate *p*H. For example, in muscles with an ultimate *p*H of 5·3, although cross-striations were still clear, the muscle fibres were frequently twisted and broken ; in those at *p*H 5·1 the protein gel in the interior of more than half of the fibres appeared to have coagulated. At *p*H 4·9 all fibres were abnormal : some of these showed no cross-striations but had manifest longitudinal markings ; others had still cross-striations but were both twisted and finely corrugated (Fig. 1). The general histological picture resembles that found in isolated muscle where *rigor mortis* has been precipitate due to an abnormally high rate of breakdown of adenosinetriphosphate (Bendall, J. R., personal communication). This aspect of the question is being studied further.

It is hoped to publish a fuller account of these investigations elsewhere.

The technical assistance of Messrs. W. A. Deer and N. King is gratefully acknowledged. Samples of

pig muscle were kindly provided by Messrs. Robert Lawson and Sons (Dyce) Ltd.

This work has been carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

R. A. Lawrie
D. P. Gatherum
H. P. Hale

Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge and Department of Scientific and Industrial Research.

July 17.

[1] Callow, E. H., Rep. Food Invest. Bd., 34 (1937).
[2] Lawrie, R. A., *J. Physiol.*, 121, 275 (1953).
[3] Howard, A., and Lawrie, R. A., Spec. Rep. No. 63, Food Invest. Bd. (H.M. Stationery Office, 1956).
[4] Callow, E. H., Rep. Food Invest. Bd., 31 (1939).
[5] Ludvigsen, J., 272 ber fra forsøgslaboratoret (Copenhagen, 1954).
[6] Henry, M., Billon, J., and Haouza, G., *Rev. Path. Gen. Comp.*, No. 669, 857 (1955).
[7] Henry, M., Romani, J.-D., and Joubert, L., *Rev. Path. Gen. Phys. Clin.*, No. 696, 355 (1958).

Establishment of Permanent Cell Strains from Human Adult Peripheral Blood

Cultures of cells from the buffy coat of the blood were described by Carrel and Ebeling in 1922[1], and since then they have been used by many workers. Several have described the appearance of fibrocytic cells in these cultures[2]. They have been reported as early as one day after explantation, and Hulliger[3], using mainly rabbit buffy coat, found that within one to three weeks cultures of fibrocytic cells were frequently formed in which a high level of mitosis occurred. It would be useful to be able to establish strains of cells from individuals whenever they are required for certain genetic studies, and since the peripheral blood is the easiest tissue to obtain aseptically from volunteers, the possibility that permanent strains might be developed from this source was investigated. Strains were established from four out of five bloods. The technique used is as follows.

15–20 ml. of blood are collected in sterile siliconed tubes and buffy coat cultures are prepared in 50 ml. conical flasks, essentially by the method described by Allgöwer[3]. After one week a fluid medium consisting of 20 per cent horse or calf serum in Eagle's medium[3] (with phenol red) is added to the plasma clot cultures. About this time the cultures enter a static phase in which they remain for a period which varies from a few days to many weeks. The only evidence of the presence of surviving cells is in some cases the progressive fall in the *p*H of the medium. This is observed daily, and when it falls to 7·0 the fluid phase is renewed. Quite suddenly rapidly growing colonies of cells appear. These can be trypsinized in the usual manner and established in continuous cultivation. They resemble other 'fibroblastic' strains in morphology and grow very well in 20 per cent calf serum in Eagle's medium. They can be cloned quite easily by the dilution technique[4]. The chromosomes have been identified as human by Prof. Pontecorvo and Mr. C. G. Elliott of the Genetics Department of this University.

The origin of these cells is by no means certain. The following suggestions have been made: (1) They represent transformed monocytes or lymphocytes; (2) they are cells carried into the vein during venepuncture; (3) they are tissue fibroblasts or other cells, not usually listed among the leucocytes, which exist in small numbers in the peripheral blood. In view of the regularity with which they can be obtained, I feel that the second explanation is unlikely. But since their origin is at present uncertain they are best described as normal adult human buffy coat cells. We have four strains, HERT 1, HERT 2, HERT 3 and HERT 4, isolated from four different individuals and maintained in continuous culture for periods varying from six months to a year, with a minimum of thirty serial passages.

If these are normal blood elements, no matter what their precise origin, it seems that Allgöwer's suggestion that they give rise to the fibroblasts of granulation tissue has some justification. Also it seems not unlikely that many of the 'fibroblastic' strains of cells isolated from a variety of tissues may, in fact, have their origin from these blood cells. Finally, in view of the readiness with which the colonies grow from individuals the danger of using unfiltered serum in culture media is obvious.

John Paul

HERT Tissue Culture Laboratory,
Department of Biochemistry,
University of Glasgow.

June 26.

[1] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 36, 365 (1922).
[2] Maximow, A. A., *Arch. Exp. Zellforsh.*, 5, 169 (1928). Ehrich, W. E., *Erg. Path.*, 29, 1 (1934). Allgöwer, M., "The Cellular Basis of Wound Healing" (Charles Thomas, Springfield, Ill., 1956). Hulliger, L., *Virchow's Archiv.*, 329, 289 (1956).
[3] Eagle, H., *J. Exp. Med.*, 102, 595 (1955).
[4] Puck, T., Marcus, P. I., and Cieciura, S. J., *J. Exp. Med.*, 103, 273 (1956).

Protoplasts of Group A Beta-hæmolytic Streptococci

This communication records the preparation from strains of group *A* streptococci of bodies which appear to conform to the rigid definition of 'protoplasts' recently offered by Brenner *et al.*[1]. They have been prepared by enzymic digestion of living cells.

There are two enzyme systems which attack isolated cell walls of group *A* streptococci: the *Streptomyces albus* mixture[2] and the enzyme produced during bacteriophage lysis of group *C* streptococci[3]. Both enzyme systems have been used successfully to prepare protoplasts, but the latter enzyme system is the more suitable since the streptomyces mixture contains an enzyme(s) which attacks the protoplast membrane.

Our general method has been to incubate the streptococcus (Lancefield group *A*, type 14 concentration 10^8 orgs./ml.) with the enzyme in 2 *M* sodium chloride or 2 *M* sucrose for 30 min. at 37° C. Little change in turbidity, as measured spectrophotometrically, occurs in this time and microscopical examination of air-dried stained smears of the suspension shows discrete spherical particles. Under dark-ground illumination they are indistinguishable from the original streptococcus (except possibly smaller). The suspension is centrifuged, the precipitate washed twice with 2 *M* salt solution and resuspended in a medium of suitable composition for further studies. The properties of these protoplasts, are as follows:

Osmotic sensitivity. Suspension of the protoplasts in distilled water or dilute (less than *M*) buffer brings about immediate lysis with the release of internal components of the organism. Under appropriate

conditions highly viscous preparations possibly containing polymerized nucleoproteins can be prepared. Centrifugation of the lysed material at 20,000 r.p.m. for 15–30 min. gives a small pellet of insoluble opaque residue.

Immunochemistry. The protoplasts can be shown to be free of antigenic substances normally believed to occur in the cell wall. The precipitating *M* antigen and agglutinating *T* antigen, together with the group hapten, are absent when tested for by conventional typing methods. Antisera prepared against both whole and fractionated protoplasts have failed to show evidence of antibody to any of the known cell wall components.

Chemistry. Rhamnose has been shown to occur almost exclusively in the cell wall of the group *A* streptococcus. In the process of protoplast formation the rhamnose is liberated in a soluble form, which is still serologically active as group hapten. Washed protoplasts have been shown to contain less than 10 per cent of the rhamnose present in a similar sample of washed streptococci treated with inactivated enzyme. (This is a minimum figure since no account was taken of any lysis which must have occurred on washing.)

Interaction with bacteriophage. Intact cells readily adsorb appropriate bacteriophage whereas protoplasts of the same strain fail to do so.

Growth of protoplasts. Many attempts under varying conditions have been made to induce the protoplasts to grow or synthesize new cell wall material. No success has yet been attained.

Behaviour of isolated cell walls. Isolated cell walls do not appear to undergo complete dissolution when treated with partially purified enzyme preparations. Group hapten is liberated (in some cases quantitatively) but an insoluble residue remains. None of the components liberated by the partially purified enzyme appears to be dialysable.

These properties show that the enzyme treatment has removed the cell-wall components from the streptococci, and that the osmotically fragile structures produced can justifiably be called protoplasts. These protoplasts of hæmolytic streptococci are similar structures to those produced by the action of lysozyme on *B. megaterium*.

H. Gooder
W. R. Maxted

Central Public Health Laboratory,
Colindale, London, N.W.9.
Aug. 1.

[1] Brenner, S., *et al.*, *Nature*, **181**, 1713 (1958).
[2] McCarty, M., *J. Exp. Med.*, **96**, 569 (1952).
[3] Maxted, W. R., *J. Gen. Microbiol.*, **16**, 584 (1957). Maxted, W. R., and Gooder, H., *ibid.*, **18**, xiv (1958). Krause, M., *J. Exp. Med.*, **106**, 365 (1957).

Calcium Exchanges of Ehrlich Ascites Tumour Cells of Mice *in vitro*

In a previous communication we reported the observation that when the isotope calcium-45 was injected intraperitoneally into mice bearing Ehrlich ascites tumours, the specific activity of the calcium of the cells had already reached that of the fluid after the shortest interval allowed between injection of the isotope and sampling the tumour (4 min.).

Subsequent experiments have confirmed this result, and we are now studying calcium exchange *in vitro*. In a total of six experiments in which cells labelled with calcium-45 *in vivo* were incubated *in vitro* for 10 min. at 37° C. suspended in unlabelled ascitic fluid taken previously from the same mouse, no significant exchange was found to take place between the calcium-45 of cells and fluid. The same result was obtained in five experiments in which unlabelled cells were incubated with ascitic fluid containing calcium-45. Preliminary experiments in which cells were incubated in ascitic fluid at 37° C. for up to 80 min. in the presence of calcium-45 indicate that, even for this period, exchange is so slow as to be negligible.

These results suggest that calcium exchange, which *in vivo* seemed so rapid and complete as to be plausibly explicable in terms of diffusion alone, in fact must depend on the metabolic condition of the cell.

We are at present studying the exchange of calcium by cells suspended in a nutrient, balanced medium, in the hope of imitating and investigating further the process which occurs *in vivo*.

Doreen Thomason
Raymond Schofield

Christie Hospital and Holt Radium Institute,
Withington,
Manchester, 20.

[1] Thomason, D., and Schofield, R., *Nature*, **181**, 1207 (1958).

Amœboid Motility of Fresh Ascites Tumour Cells

Using the fresh ascitic fluid of ascites tumours, and no other medium, I have studied the amœboid motility of living tumour cells. The Ehrlich ascites carcinoma and three strains of the ascites hepatoma, *AH* 130, *AH* 602 and *AH* 7974, which are transplantable ascites carcinomas of the rat established by Yoshida[1], were used. The method was as follows. A small amount of ascitic fluid was withdrawn at any time from the peritoneal cavity of host animals by means of a fine, sterile glass pipette. One drop of fresh undiluted ascitic fluid was immediately placed on the centre of a clean cover-slip, forming a globule about 5 mm. in diameter. The excess ascitic fluid was removed with the glass pipette to leave a thin flat covering of fluid. The cover-slip was then inverted over a depression slide having a depression 15 mm. in diameter and 0·5 mm. in depth, around the rim of which sufficient vaseline had been smeared to act as a seal. A slight pressure was applied to the cover-slip to seal it completely with the slide. The hanging-drop preparation was then ready for microscopic observation. The observations were carried out at 37° C. in a chamber equipped with an electric warmer.

As a rule, one hour after preparation, tumour cells of each strain became actively amœboid. In time, most of the Ehrlich ascites carcinoma cells showed amœboid movement, becoming roughly fusiform with pseudopods (Fig. 1). The ascites hepatoma is not a free-cell tumour in the strict sense of the word, as was pointed out by Yoshida[1], that is, the tumour cells, being epithelial in nature, make cell associations ('islands') suspended in the ascitic fluid, and individually isolated tumour cells are also present. In the ascites hepatoma, with the exception of *AH* 602, isolated tumour cells displayed amœboid movement, and individual tumour cells forming islands were also observed to move with pseudopods (Fig. 2). Accord-

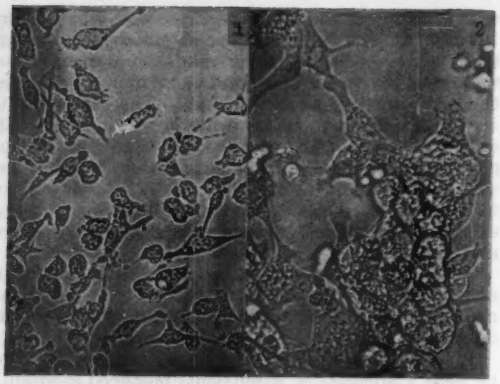


Fig. 1. Ehrlich ascites carcinoma, 6 hr. preparation. (× 135)
Fig. 2. Ascites hepatoma 130, 6 hr. after preparation. (× 270)

ing to my observations, tumour cells usually displayed amœboid movement actively for about six hours or more without showing any signs of degenerative cells. Intermingled with tumour cells, a few polymorphonuclear leucocytes, lymphocytes and macrophages were also seen. However, these could easily be distinguished from the tumour cells not only by their shape and size but even more accurately by the speed and character of their movement as reported by Enterline and Coman[2]. The alterations in positions of the estimated centre of the nucleolus of each tumour cell were traced on paper at five-minute intervals using Abbe's drawing camera. In this way, rates of amœboid movement were calculated in μ/min. (Table 1). Almost all tumour cells of *AH* 602 showed no movement with this recording technique. The average rate of movement of the Ehrlich ascites carcinoma and *AH* 7974 was 0·9 μ/min. and that of *AH* 130 was 1·0 μ/min. The difference of the average rates of movement of tumour cells among these three strains was not statistically significant. The maximum rate was calculated in μ/min. from the greatest distance any cell moved in a five-minute interval (Table 1). While tumour cells in *AH* 130 and *AH* 7974 almost always showed amœboid movement, cells in *AH* 602 did so very rarely. It is interesting that the motility should thus depend on the strain of hepatoma. Furthermore, it may be deduced that ascites tumour cells display amœboid movement in contact with the peritoneum *in vivo* too, and that they are most likely to invade in regions of the peritoneum where fluid stagnation can take place, that is, where tumour cells remain in contact with the peritoneum for some time, as Baillif[3] observed histologically, using mice inoculated with the Ehrlich ascites carcinoma.

I wish to express my sincere thanks to Prof. T. Yoshida for supplying the ascites hepatoma, and to Mr. Z. Aoki for his assistance during the course of this work.

Table 1. Rates of Amœboid Movement of Ehrlich Ascites Carcinoma Cells and Ascites Hepatoma Cells

	No. of cells observed	Average rate (μ/min.) Mean ± S.E.	Maximum rate (μ/min.)
Ehrlich ascites carcinoma	19	0·9 ± 0·1	2·2
Ascites hepatoma 130	14	1·0 ± 0·1	2·2
Ascites hepatoma 7974	15	0·9 ± 0·1	2·8

Iwao Hirono

Department of Pathology,
Nagoya University School of Medicine,
Japan.
July 15.

[1] Yoshida, T., *Ann. N.Y. Acad. Sci.*, **63**, 852 (1956).
[2] Enterline, H. T., and Coman, D. R., *Cancer*, **3**, 1033 (1950).
[3] Baillif, R. N., *Cancer Res.*, **14**, 554 (1954).

Cultivation of Isolated Embryonic Rat Tibiæ on a Chemically Defined Medium

The successful culture of embryonic chick femora and tibiæ on a chemically defined medium has recently been described[1]. The medium used was No. 858[2], which was originally designed for the culture of Earle's L-strain cells. We have now been able to cultivate embryonic rat tibiæ on a closely related, chemically defined medium (J. D. Biggers and J. A. Lucy, unpublished work), thus paving the way for future studies on the specific nutritional requirements and metabolism of growing mammalian cartilage.

The bones have been cultured by means of the floating-lens paper technique devised by Chen[3]. Each watch-glass contained 0·5 ml. of medium and this was used for the cultivation of one tibia, the medium being renewed every second day. Rat fœtuses of a known age were obtained from the Medical Research Council strain of albino rats bred in the Department. The occurrence of mating was determined by finding a vaginal plug, the day on which the plug was found being counted as the first day of pregnancy. On the seventeenth day of pregnancy the uterus and conceptuses were excised. The fœtuses were immediately freed from their fœtal membranes and placed in fresh sterile Tyrode's solution at room temperature until the tibiæ could be isolated. In previous experiments it was found that the growth of the tibiæ was poor when the bones were isolated from fœtuses which had remained in the isolated uterus. The tibiæ were removed under a dissecting microscope and cleaned of as much extraneous tissue as possible, leaving the perichondrium intact.

Tibiæ from 17-day-old rat fœtuses may vary between approximately 1 and 3 mm. in length. This variation is mainly between pregnant rats, and may be accounted for largely in terms of the variation between individual rats in the number of embryos *in utero*. The shorter bones are composed of hyaline cartilage with intact hypertrophic cells in the centre of the diaphysis. The longer bones possess, in addition, a narrow collar of ossification in the diaphysis and, further, the central hypertrophic cells may have started to degenerate as a preliminary to endochondral ossification.

Pilot experiments showed that 17-day-old embryonic rat tibiæ elongate when they are cultivated on the chemically defined medium. Since previous work with embryonic chick long bones demonstrated that the degree to which bones elongate depends considerably on the level of glucose which is originally present in the medium[1,4] an experiment was designed to examine the effect of glucose on the elongation of rat tibiæ. Six batches of media were prepared, containing 0·5, 1, 2, 4, 8 and 16 mgm./ml. glucose respectively. These glucose levels form a set of six treatments which

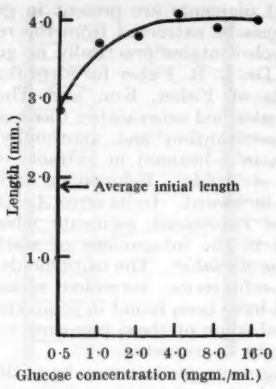


Fig. 1. The effect of glucose concentration on the length of 17-day-old embryonic rat tibiæ cultivated for five days on a chemically defined medium. Each point is the mean of six observations

were replicated six times in a randomized block design. Three pregnant rats were used, each providing the tibiæ for two blocks, and the tibiæ forming each block were allocated to the six treatments by means of a table of random numbers. Thus the comparison of the glucose levels is made within pregnant rats with a considerable gain in precision. The bones were drawn daily by means of a camera lucida, and the lengths of the bones at different stages were calculated from these drawings. Standard statistical methods were used to analyse this experiment. The results were transformed to the logarithmic scale before analysis.

The effect of concentration of glucose on the length of the bones after 5 days cultivation is shown in Fig. 1. The dose-response line was calculated after allowing for variations in initial length by an analysis of covariance. The results show that a glucose-level of less than 3 mgm./ml. is insufficient to promote maximum elongation. With higher glucose-levels the dose-response line becomes horizontal, indicating that glucose has no deleterious effects in concentrations up to 16 mgm./ml. This finding suggests that, as in the case of embryonic chick tibiæ, glucose penetrates the cells so quickly that the osmotic action of high concentrations is transitory, with no permanent effects on the subsequent growth of the bones. The elongation observed when the concentration of glucose in the medium is greater than 3 mgm./ml. was 112 per cent. Fig. 2 shows the type of growth which is obtained under these conditions. In some specimens the morphology remains approximately normal, and in others, curvature of the diaphysis may develop.

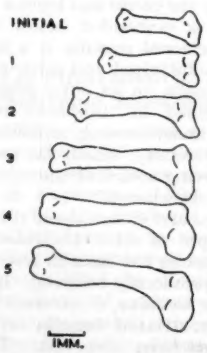


Fig. 2. Daily camera lucida drawings of a 17-day-old embryonic rat tibia cultivated on chemically defined medium (glucose concentration, 4 mgm./ml.). The medium was renewed on the second and fourth days

A second experiment was carried out to examine the histology of embryonic rat tibiæ grown on a chemically defined medium, containing 4 mgm./ml. glucose. Twenty tibiæ were explanted and at daily intervals four of these were chosen at random and fixed in Zenker containing 3 per cent acetic acid. Sections of these bones were stained with Ehrlich's hæmatoxylin and erythrosin and also with Heidenhain's hæmatoxylin. The bones remained healthy up to 4 days. The cells of the epiphysis and diaphysis retained their normal morphology, and the nuclei of these cells often contained two or three clearly defined nucleoli. Mitoses were easily found in the cells of the epiphysis up to the third day. At five days some of the hypertrophic cells of the diaphysis were degenerate, although the epiphyseal cells were still normal.

We have also attempted to culture 18-day embryonic mouse tibiæ on a chemically defined medium. The elongation which was obtained was of a similar magnitude to that recorded by Fell and Mellanby[5] using a natural medium. The elongation, however, was relatively small in comparison with that obtained with 17-day rat tibiæ. The difference in growth in the two cases is probably related to the difference in the initial structure in the tibiæ when they are explanted. In the rat, very little or no ossification of the diaphysis has occurred, and elongation is caused by hypertrophy of the diaphyseal cells, proliferation of the epiphyseal cells and secretion of new matrix. At this stage hypertrophy of the diaphyseal cells plays a major role in the elongation of the tibiæ which occurs *in vivo*[6]. In the mouse, on the other hand, there is considerable ossification of the diaphysis so that elongation cannot result from hypertrophy of diaphyseal cells, but largely depends on the proliferation of epiphyseal cells and the secretion of matrix between them. It would be interesting to explant mouse tibiæ at a stage of development comparable with that of 17-day-old embryonic rat tibiæ but, unfortunately, this has not been possible for technical reasons.

We wish to thank Mr. John Roberts for skilled technical assistance. Also we wish to acknowledge generous grants from the Royal Society, the Nuffield Foundation, the Medical Research Council and the Central Research Fund of the University of London, which made this work possible.

SUSAN HEYNER
J. D. BIGGERS

Department of Physiology,
Royal Veterinary College,
London, N.W.1.
June 11.

[1] Biggers, J. D., Webb, M., Parker, R. C., and Healy, G. M., *Nature*, **180**, 825 (1957).
[2] Healy, G. M., Fisher, D. C., and Parker, R. C., *Proc. Soc. N.Y. Exp. Biol.*, **89**, 71 (1955).
[3] Chen, J. M., *Exp. Cell. Res.*, **7**, 518 (1954).
[4] Biggers, J. D., Rinaldini, L. R., and Webb, M., *Symp. Soc. Exp. Biol.*, **11**, 264 (1957).
[5] Fell, H. B., and Mellanby, E., *J. Physiol.*, **116**, 320 (1952).
[6] Bhaskar, S. N., Weinmann, J. P., and Schour, I., *Anat. Rec.*, **119**, 231 (1954).

Pigments Present in Arms and Pinnules of the Crinoid, *Antedon bifida* (Pennant)

In *A. bifida* red pigment usually occurs in protein granules in superficial connective tissue and in the eggs. The skeleton is colourless. When freshly detached pinnules are placed on slides and covered with sodium hydrosulphite solution, microscopic examination reveals that the red colour is immediately lost, but is restored in the granules when this solution is replaced quickly by sea water, and more markedly if drops of hydrogen peroxide are added to the sea water. Cannan[1] applied this test to *Arbacia* and *Echinus* echinochrome with similar results. The pigment restored in colour passes gradually into the protein pyriform bodies in the sacculi in *Antedon*. In distilled water the red pigment also passes into these bodies, which are discharged. Ball[2] used acid alcohol to extract echinochrome from *Arbacia*, and this solvent proved to be effective in removing selectively the red pigment from *Antedon* from Plymouth. Extraction procedure and tests performed were as follows:

1. Living arms and pinnules of 10 animals were cut up into 50 ml. of acid alcohol (70 per cent methyl alcohol containing 0·75 per cent conc. hydrochloric acid). Pigment extracted was placed in a separating funnel.

2. 50 ml. of petroleum ether and later 60 ml. of distilled water were added to the extract, which was shaken so that carotenoids and lipids might pass into the epiphase.

3. The hypophase containing the red pigment was run into a further separating funnel. About 20 ml. of distilled water of *p*H 5 (from a metal still) and 50 ml. of diethyl ether were added. The red pigment passed into the epiphase. If 20 ml. of glass distilled water is used instead, the red pigment remains in the hypophase, but on addition of a little hydrochloric or acetic acid it rises into the ether. In this instability the pigment resembles the hydroxy-naphthaquinone extracted by Ball and Cooper[3] from *Arbacia*, and by Millott[4,5] from *Diadema antillarum*; furthermore it is orange in aqueous acid solution and deep red in alkaline. A green colour, indicative of a phenol, appears if ferric chloride solution is added to a sample of this pigment in distilled water acidified with hydrochloric acid.

4. Samples of the ethereal solution of pigment dried with anhydrous sodium sulphate were examined in a Beckman spectrophotometer.

Absorption peaks occur at 262 mμ, 337 mμ, 460 mμ and there is an inflexion at 510 mμ—all as in the naphthaquinone pigment from *Diadema*[4,5] except that the maxima at 337 and 460 mμ are less well marked.

Yellow-brown lipid granules (which take up sudan dyes) are present in deposits in connective tissue and in coelomocytes. The brown colour is not removed by changes of acetone or ethylene chlorohydrin. In sections these granules blacken with alkaline silver methenamine. Since 0·13 per cent *l*-tyrosine is blackened slowly by ground arms and pinnules, some tyrosinase is possibly present in them, but use of the Hueck and Long Ziehl–Neelsen tests (see Pearse[6]) indicate that the yellow-brown material contains lipofuscin (oxidized lipid), not melanin, and bleaching tests bear this out, for very little brown colour is removed when sections 7μ thick are placed in 30 vol. hydrogen peroxide or 1 per cent bromine water for 48 hr.

Carotenoid pigments are present in gut wall and gonad, but can be extracted from the region of the arm-tip, which contains practically no gonad. I am indebted to Dr. L. R. Fisher for identification (using the methods of Fisher, Kon and Thompson[7]) of β-carotene, esterified astaxanthin (peak at 465 mμ in *n*-hexane), astaxanthin and xanthophyll (peak at 445–447 mμ in *n*-hexane) in extracts of arms and pinnules of *A. bifida*. Echinenone and vitamin A appeared to be absent. In its arms *A. bifida* seems to have several carotenoid pigments which resemble those found in the integument of starfish such as *Marthasterias glacialis*[8]. The carotenoids, α-carotene, β-carotene, echinenone, esterified astaxanthin and xanthophyll have been found in echinoderms and the occurrence of some of these pigments in the crinoid *A. bifida* might be expected.

Certain pigments present may be of dietary origin. *A. bifida* is both a detritus and suspension feeder. Small copepods are ingested with detritus. Diatoms are digested.

Like the echinoid *Diadema*[9], this crinoid responds to light. Animals so placed that aboral cirri rest on the bottoms of bowls will stay still in very dim light for half an hour, but rise up and swim when exposed to 160 ft. candles of light from an electric bulb for 4 sec. Preliminary work indicates that this response is also given when green, but not when blue, yellow or red filters are placed in front of the light; a light-sensitive pigment may be present in the nervous system.

E. J. Dimelow

Department of Zoology,
University of Reading.
Aug. 8.

[1] Cannan, R. K., *Biochem. J.*, **21**, 184 (1927).
[2] Ball, E. G., *J. Biol. Chem.*, **114**, vi (1936).
[3] Ball, E. G., and Cooper, O., *Biol. Bull. Woods Hole*, **97**, 231 (1949).
[4] Millott, N., *Nature*, **178**, 1185 (1956).
[5] Millott, N., *Proc. Zool. Soc. Lond.*, **129**, 263 (1957).
[6] Pearse, A. G. E., "Histochemistry" (London, 1954).
[7] Fisher, L. F., Kon, S. K., and Thompson, S. Y., *J. Mar. Biol. Assoc. U.K.*, **35**, 41 (1956).
[8] Vevers, H. G., and Millott, N., *Proc. Zool. Soc. Lond.*, **129**, 75 (1957).
[9] Millott, N., *Phil. Trans. Roy. Soc.*, B, **238**, 187 (1954).

The Finer Structure of the Galeal Lamella in Adult Mosquitoes

The maxilla of the adult mosquito is a reduced structure in that the cardo and lacinia are almost lost, and the stipes is embedded below the alimentary canal. The functional maxilla is a lancet-like galea consisting of a chitinized rod with a trough-shaped membranous lamella on its outer edge. This lamella has been described in *Anopheles maculipennis* as possessing fine anastomosing striations and bearing at its distal extremity small, backwardly pointing teeth[1]. The piercing action of the maxillary galea has been described elsewhere[2].

Studies being carried out upon the detailed structure of the trophi of adult Culicidae have revealed that in many species the lamella has a more complex structure than previously believed. In *Aedes argenteoventralis*, *Culex horridus*, *C. cinereus* and *C. fatigans*, a very fine non-striated lamella on the inner side of the galea has been observed. The structure of the outer lamella has particularly been studied in *Aedes aegypti*, *A. grahami*, *Culex nebulosus*, *C. cinerellus*, *C. fatigans*, *Uranotaenia bilineata* and *Hodgesia*

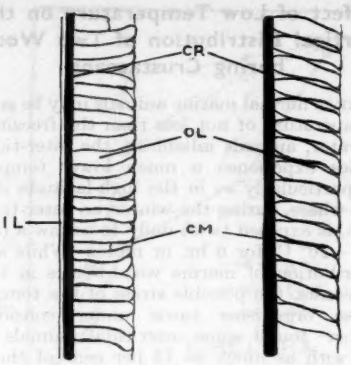


Fig. 1. Sections of the maxillary galeæ in (*a*) *Culex fatigans* and (*b*) *Aedes grahami*. *OL*, Outer lamella; *IL*, inner lamella; *CR*, chitinized rod; *CM*, connecting membrane

nigeriae. In these species the lamella has the appearance of being composed of a great number of finger-like projections arising from the chitinized rod, which are separate at their distal extremities (Fig. 1). Normal pressure on a coverslip over the portion of the lamella under observation does not cause separation of the individual parts, but when the lamella is broken these can be observed. Although these projections are considered to be separate entities, it has been observed that they are connected to one another by a much finer continuous membrane. Examination of longitudinal sections of the proboscis reveals that these projections are indeed separable when the galea is cut at an oblique angle while still in the labial sheath.

The structure of the lamella as described here has been observed in a number of widely separate species, and it is doubtful whether this type of structure is related to a particular feeding habit. Nevertheless, the possession of a lamella with a serrated, or semi-serrated, edge would be of value to a species which is a blood feeder and must therefore be able to pierce animal tissue.

G. Surtees

West African Council for Medical Research,
Lagos, Nigeria.

[1] Robinson, G. C., *Parasitology*, **31**, 212 (1939).
[2] Gordon, R. M., and Lumsden, W. H. R., *Ann. Trop. Med. Parasit.*, **33**, 259 (1939).

Dependence of Mitochondrial Swelling on Oxidizable Substrates

That the swelling of isolated mitochondria induced by various agents fails to occur under anaerobic conditions[1,2] suggested to us that swelling may be dependent on respiration. The probability of this is increased by the finding[3,4] that malonate prevents the swelling induced by succinate of mitochondria suspended in mannitol or sucrose solutions. We have therefore studied the effects of addition of substrates and inhibitors of oxidative metabolism on the swelling of mitochondria under various conditions. Mitochondria from livers of male rats (180–280 gm. body-weight) were prepared, suspended and stored in 0·44 *M* sucrose at 0° C. 0·05 ml. of mitochondrial suspension (25 mgm.-equivalents; 0·1 mgm. nitrogen) was added to 2·95 ml. of medium containing 2-amino-2-hydroxymethylpropane-1 : 3-diol (*tris*) chloride buffer, *p*H 7·5, and sucrose (final concentrations 25 m*M* and 0·3 *M* respectively). The optical density at 520 mμ (D_{520}) was recorded for periods up to 1 hr., the temperature being thermostatically controlled at 20°. Decreases in D_{520} were taken as a measure of swelling. Substrates were used in 1 m*M* concentration.

In contrast to the experience of previous workers[3,4], negligible swelling occurred during 1 hr. incubation in standard medium containing no further additions, even at 25° C. or after storage of the mitochondria at 0° C. for 26 hr.

With fresh mitochondria, the swelling caused by phosphate (10 m*M*) was increased in extent by addition of D-β-hydroxybutyrate, succinate, L-glutamate, L-malate or L-proline. These substrates, in absence of phosphate, produced negligible swelling. The effect of phosphate alone decreased as the age of the mitochondria increased. It was negligible after 18 hr., and was then restored by addition of the above substrates, which again had no effect when added alone. Pyruvate and α-oxoglutarate were, however, ineffective under these conditions.

The swelling of day-old mitochondria in the presence of succinate and phosphate ('succinate – phosphate swelling') was inhibited by malonate (1 m*M*) (Fig. 1), whereas the D-β-hydroxybutyrate – phosphate and L-glutamate – phosphate swelling was not. 1·8 m*M* amytal (5-ethyl-5-*iso*amylbarbiturate) prevents the oxidation by mitochondria of substrates

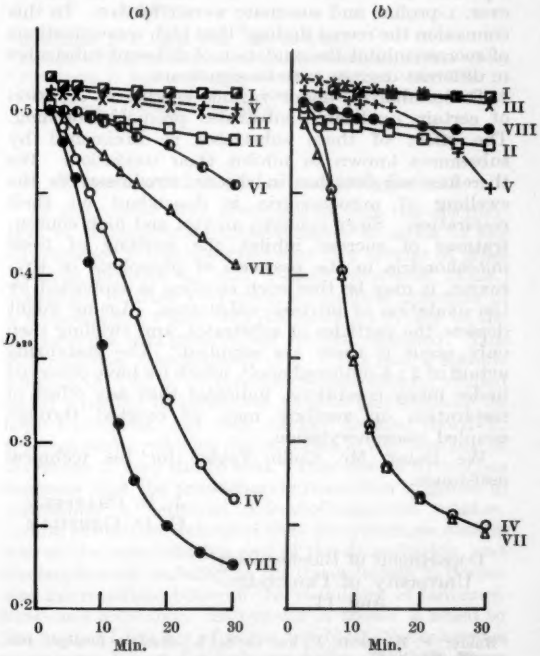


Fig. 1. Inhibitory effects of malonate and amytal on substrate – phosphate swelling in aged (23 hr.) mitochondria. Each cell contained 0·3 *M* sucrose and 25 m*M* *tris* chloride buffer, *p*H 7·52, with the following further additions, the substrate being 1 m*M* succinate in (*a*) and 1 m*M* D-β-hydroxybutyrate in (*b*): I, none; II, 10 m*M* phosphate; III, substrate; IV, substrate + phosphate; V, substrate + 1 m*M* malonate; VI, substrate (succinate only) + 2 m*M* amytal; VII, substrate + phosphate + malonate; VIII, substrate + phosphate + amytal

with diphosphopyridine nucleotide-linked dehydrogenases, but has no effect on the oxidation of succinate[5]. It is therefore of interest that 1·8 or 2 m*M* amytal inhibited the swelling caused by D-β-hydroxybutyrate, L-glutamate, L-malate or L-proline when added together with phosphate, but not the succinate – phosphate swelling (Fig. 1). Antimycin *A* (0·05 μgm./ml.), which blocks the respiratory chain[6], inhibited by 50–100 per cent the swelling induced by all the above substrates, including succinate, in presence of phosphate. Cyanide (1 m*M*) abolished the D-β-hydroxybutyrate – phosphate swelling of fresh and aged mitochondria.

Studies of the swelling induced by thyroxine have provided similar evidence that swelling may be dependent on the presence of oxidizable substrates. Thus, with fresh mitochondria, D-β-hydroxybutyrate enhanced, and with aged mitochondria restored, the thyroxine effect. D-β-Hydroxybutyrate – thyroxine swelling was inhibited by amytal and antimycin *A*. Still further evidence has been provided by the observation that with day-old particles in 0·2 *M* sucrose buffered with 25 m*M* *tris*, swelling was induced by succinate, D-β-hydroxybutyrate and L-glutamate. Malonate strongly inhibited the effect of succinate but accelerated that of the other two substrates.

With fresh mitochondria, replacing the *tris* buffer by glyoxaline or raising the sucrose concentration from 0·3 *M* to 0·5 *M* prevented swelling due to phosphate. This could then be restored by addition of D-β-hydroxybutyrate but not of L-glutamate, L-proline or succinate. With 0·45 *M* sucrose, however, L-proline and succinate were effective. In this connexion the recent finding[7] that high concentrations of sucrose inhibit the oxidation of different substrates in different degrees may be significant.

To summarize, under various conditions addition of certain oxidizable substrates promotes swelling. The effect of these substrates is diminished by substances known to inhibit their oxidation. We therefore suggest that in certain circumstances the swelling of mitochondria is dependent on their respiration. Since cyanide, amytal and high concentrations of sucrose inhibit the swelling of fresh mitochondria in the presence of phosphate or thyroxine, it may be that such swelling is supported by the oxidation of intrinsic substrates. Ageing might deplete the particles of substrates, and swelling then only occur if these are supplied. The stabilizing action of 2 : 4-dinitrophenol[4], which we have observed under many conditions, indicates that any effect of respiration on swelling may be exerted through coupled phosphorylation.

We thank Mr. Colin Taylor for his technical assistance.

J. B. Chappell
G. D. Greville

Department of Biochemistry,
University of Cambridge.
Aug. 11.

[1] Hunter, F. E., Davis, J., and Carlat, L., *Biochim. Biophys. Acta*, **20**, 237 (1956).
[2] Lehninger, A. L., and Ray, B. L., *Biochim. Biophys. Acta*, **26**, 643 (1957).
[3] Rafaiub, J., *Helv. Physiol. Pharmacol. Acta*, **11**, 142 (1953).
[4] Tapley, D. F., *J. Biol. Chem.*, **222**, 325 (1956).
[5] Ernster, L., Jalling, O., Löw, H., and Lindberg, O., *Exp. Cell Res.*, Supp. **3**, 124 (1955).
[6] Potter, V. R., and Reif, A. E., *J. Biol. Chem.*, **194**, 287 (1952).
[7] Johnson, D., and Lardy, H. A., *Nature*, **181**, 701 (1958).

Effect of Low Temperature on the Vertical Distribution of Two Wood-boring Crustaceans

Whereas normal marine animals may be subjected to a temperature of not less than the freezing point of sea water, animals inhabiting the inter-tidal zone sometimes experience a much lower temperature. This is particularly so in the high-latitude shores of Norway where, during the winter, an inter-tidal zone of 3·5 m. is exposed twice daily to as low a temperature as −20° C. for 6 hr. or more. While studying the distribution of marine wood-borers in the Norwegian coasts, the possible stress of low temperature on these organisms came under consideration. Kanwisher[1] found some inter-tidal animals able to survive with as much as 75 per cent of their body fluids converted as ice. In *Limnoria*, Sømme[2] found that 83 per cent died when frozen in ice for 2·5 hr. With the view of finding out the extent of tolerance and any evidence of an adaptability to sudden drops in temperature which these animals experience when they are exposed to the air by the receding tide, the following experiments were performed.

Sectors approximately 50 cm. × 15 cm. × 5 cm. in size infected by borers obtained from nearby piles, were used in the experiments. These were acclimatized in a submerged condition from +6° C., the temperature of the surrounding water, to +2° C. during the course of 24 hr., after which they were exposed to air of different low temperatures in thermostatically controlled chambers where the variation during the course of any experiment was not more than ±2° C. After definite intervals of time, approximately 25 cm.[2] of the superficial part of a block, with its contained animals, was sawn out and transferred to sea water at +2° C. where it was left for 12 hr. for recovery from exposure. After this treatment, the piece of wood was carefully dissected and the animals in it released into fresh sea water and watched for signs of life under a binocular microscope. Those that showed no signs of movement even after gentle teasing were counted as dead. Several series were run, each involving more than 500 animals, in order to allow for individual variation.

In these experiments an effort has been made to reproduce so far as possible the actual conditions experienced by these animals in Nature, thereby avoiding the artificial conditions of earlier observations (ref. 2, p. 153 and ref. 3) where animals were removed from their burrows, kept in water and subjected to low temperatures. However, the present procedure introduces an uncertainty regarding the actual temperature experienced by the animal in the burrow. Therefore, to give an idea of the rate of cooling of the log, a test run at −6° C. was carried out which showed that, at a depth of 2 cm., which is approximately the level to which *Limnoria* penetrates, the ambient temperature was reached within 3 hr. The results of the experiments are shown graphically in Fig. 1.

The three experimental periods presented in the graph represent three different exposure-periods to which *Limnoria lignorum* (Rathke) and *Chelura terebrans* (Philippi), occupying different positions on a pile in the inter-tidal zone, experience during the recession of the tide. Assuming 50 per cent mortality as a limit of tolerance of the population under consideration, it will be seen that a temperature of −4° C. is of no serious effect on *Limnoria*. On the

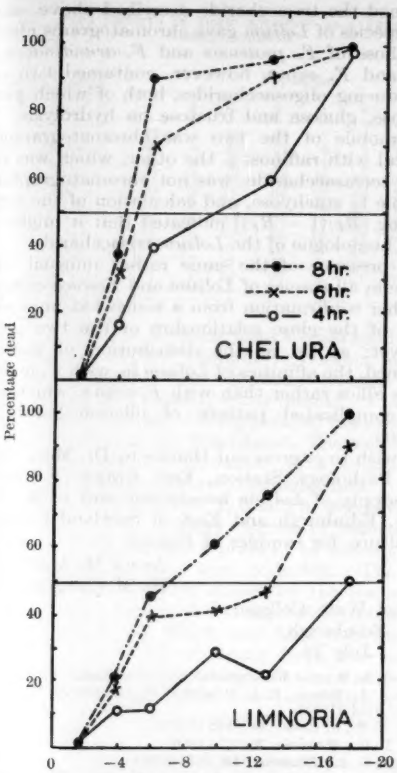


Fig. 1. Curves illustrating the death of *Chelura* and *Limnoria*, in sectors of logs exposed to different temperatures for various exposure times

other hand, this limit is almost reached within 8 hr. for the amphipod borer. This means that only those inhabiting the higher levels above mid-water mark will be affected by this temperature. Exposure to −8° C. for a period of 8 hr. kills 50 per cent of *Limnoria*, whereas this point is nearly reached within 4 hr. for *Chelura*. The latter temperature would therefore be a serious stress on those *Chelura* living both above and below the mid-water level. For a majority of *Limnoria* living at higher levels above the low-water mark a temperature of −18° C. is detrimental, since an exposure of only 4 hr. is sufficient to kill 50 per cent.

It may be inferred, therefore, that a sudden drop of temperature to −20° C. is lethal for those animals living high up on the piles above the low-water mark. Furthermore, the whole population of the inter-tidal belt experiences the great stress of low temperature during the period of exposure. It is doubtful that an organism which is subjected to such a stress under the conditions stated will continue to live and reproduce in an environment with this stress imposed r[h]ythmically twice a day. The only escape to better conditions is by active migration to more favourable locations. It was interesting to note during the experiments that many of these animals when thawed out from a state of suspended animation showed a tendency to swim out of their burrows, suggesting that they are capable of migration. Evidently, as they emerge from their dark burrows they are influenced by photic stimuli. Since the degree of settlement has been reported as inversely proportional to increasing intensity of light[4], they move to lower levels in the same pile or adjacent piles. This would explain why empty burrows are found on piles above the low-water level after the winter months. It is noteworthy that during February, which is normally the coldest month in these localities, a secondary migration of *Limnoria* has been recorded (ref. 2, p. 202) besides the primary one, which takes place during September–October. It is not improbable that some of these February migrants are from the inter-tidal zone and have deserted it in search of a more favourable habitat.

This, then, may also explain the reported peculiarity in the vertical distribution of these borers in northern latitudes, where sub-zero atmospheric temperature is quite a normal feature during the greater part of winter. Those individuals that ascend too high up the pile would be killed, establishing an upper limit in the distribution. This limit may later be extended as re-colonization is made possible on the return of more favourable conditions and when population pressure drives individuals to unoccupied regions on the pile. The progressive decrease in the infestation of *Limnoria* from the mud-line upwards in these localities, in contrast to the condition reported from several places in the south (ref. 2, p. 151), support this view.

N. Balakrishnan Nair
H. Leivestad

Biological Station,
University of Bergen,
Espegrend, Norway.
July 12.

[1] Kanwisher, J. W., *Biol. Bull.*, **109**, 60 (1955).
[2] Somme, O. M., *Nytt Magasin for Naturvidenskapene*, **81**, 154 (1940).
[3] Henderson, J. T., *Contrib. Canadian Biol.*, N.S., **2**, 317 (1924).
[4] Isham, L. B., Smith, F. G. W., and Springer, V., *Bull. Mar. Sci. Gulf and Caribbean*, **2**, 62 (1951).

Trisaccharides of *Lolium* and *Festuca*

In the course of an investigation into the distribution of water-soluble carbohydrates in seeds of the Gramineae[1], it was observed that two species of grass (*Lolium perenne* L. and *Festuca pratensis* Huds.) contained in their caryopses substantial amounts of a trisaccharide which was not detected in any of the thirty other wild grasses and cereals examined. This non-reducing trisaccharide (Fig. 1, *X*) had a specific rotation of +90·5° (without recrystallization), yielded equal amounts of galactose, glucose and fructose on acid hydrolysis and was readily broken down to fructose and a reducing disaccharide in the presence of invertase or very dilute acid. This ease of hydrolysis suggests that the trisaccharide resembles raffinose in containing a terminal β-fructofuranoside residue. Iodine oxidation[2] indicated that the galactose residue was at the non-reducing end of the disaccharide, and electrophoretic mobility of the disaccharide in borate was intermediate between the mobilities of laminaribiose and melibiose. Movement in borate is allied to the mode of linkage of sugar residues[3], disaccharides containing 1,2 or 1,4 linkages being of much lower mobility than those containing 1,3 or 1,6 linkages. The disaccharide under consideration would therefore appear to contain either a 1,3 or a 1,6 linkage. Although the electrophoretic behaviour in borate does not distinguish between α and β linkages, the high positive rotation of the trisaccharide suggests

Table 1. OLIGOSACCHARIDES OF *Lolium* AND *Festuca*

	Sucrose	Raffinose	Other tri-saccharide	Tetra-saccharide
Lolium perenne	+	−	+	−
L. italicum	+	−	+	−
L. temulentum	+	−	+	−
Festuca pratensis	+	−	+	−
F. arundinacea	+	−	+	−
F. rubra	+	+	+	+
F. ovina				
Ssp. *ovina*	+	+	+	+
Ssp. *tenuifolia*	+	+	+	+

+, Present; −, not detected.

that the galactose and glucose residues are α-linked, and, since 6-glucose-α-galactoside is melibiose, it seems probable that the newly isolated disaccharide is 3-glucose-α-galactoside. The trisaccharide from *Lolium perenne* and *Festuca pratensis* is therefore tentatively described as α-D-galactopyranosyl-3-α-D-glucopyranosyl-2-β-D-fructofuranoside.

The presence of this rather unusual trisaccharide in two members of the Festuceae and its apparent absence from certain other members of the tribe (*Dactylis* and *Cynosurus*)[1] raises the interesting possibility of examining the biochemical affinities of different species of *Festuca* and *Lolium*. It will be recalled that recent morphological and genetical studies of these two genera have cast doubts on their traditional taxonomic alignment. Tutin[4], for example, concludes that *L. perenne* should be transferred to *Festuca*, while Stebbins[5], considering that *F. pratensis* is more closely related to *L. perenne* than it is to other species of *Festuca* such as the type species, *F. ovina*, regards the 'genus' *Lolium* as a section of the large and diverse genus *Festuca*.

The results of chromatographic analyses of the sugars of several species of *Lolium* and *Festuca* are shown in Table 1 and in Fig. 1. All species examined

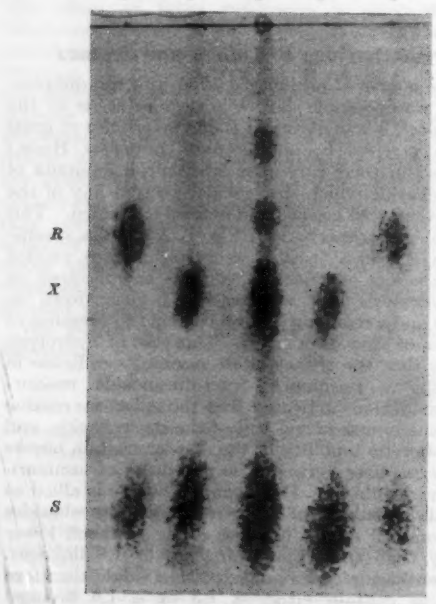


Fig. 1. Oligosaccharides of *Lolium* and *Festuca*. *C*, Control sugars; *R*, raffinose; *S*, sucrose. 1, *Lolium perenne*; 2, *Festuca rubra*; 3, *Festuca pratensis*; *X*, trisaccharide (see text). Solvent: n-propanol/water, 78/22

contained the trisaccharide described above, and all three species of *Lolium* gave chromatograms identical with those of *F. pratensis* and *F. arundinacea*. *F. rubra* and *F. ovina*, however, contained two other non-reducing oligosaccharides, both of which yielded galactose, glucose and fructose on hydrolysis. The more mobile of the two was chromatographically identical with raffinose; the other, which was probably a tetrasaccharide, was not chromatographically referable to stachyose, and calculation of the expression[6] log $[R_F/(1 - R_F)]$ indicated that it might be a higher homologue of the *Lolium* trisaccharide.

The presence of the same rather unusual trisaccharide in all species of *Lolium* and *Festuca* examined is further confirmation from a somewhat unexpected source of the close relationship of the two genera. Moreover, as far as the distribution of sugars is concerned, the affinities of *Lolium* lie with *F. pratensis* and its allies rather than with *F. ovina*, which has a more complicated pattern of oligosaccharide distribution.

We wish to express our thanks to Dr. Mary Noble, Plant Pathology Station, East Craigs, Edinburgh, for a supply of *Lolium temulentum* and to Mr. L. S. Cobley, Edinburgh and East of Scotland College of Agriculture, for samples of *Festuca*.

ANNA M. MACLEOD
H. MCCORQUODALE

Heriot-Watt College,
Edinburgh.
July 18.

[1] MacLeod, A. M., and McCorquodale, H., *New Phytol.*, **57**, 168 (1958).
[2] Barker, S. A., Bourne, E. J., Hewitt, C. C., and Stacey, M., *J. Chem. Soc.*, 3734 (1955).
[3] Foster, A. B., *J. Chem. Soc.*, 982 (1953).
[4] Tutin, T. G., *Watsonia*, **3**, 317 (1956).
[5] Stebbins, G. L., *Evolution*, **10**, 235 (1956).
[6] French, D., and Wild, G. M., *J. Amer. Chem. Soc.*, **75**, 2612 (1953).

Populations of *Agrostis* spp. Tolerant of Heavy Metals

A NUMBER of collections of *Agrostis tenuis* and *A. stolonifera* have been obtained from sites contaminated by heavy metals. Some of these have been tested for tolerance of four metals, copper, nickel, zinc and lead, using as controls populations from more normal habitats. The method used in testing is derived from that of Wilkins[1]. The amount of root growth in solutions containing the heavy metals is expressed as a percentage of that in solutions containing none. All culture solutions contained 1 gm./l. hydrated calcium nitrate. The copper and lead were present as nitrates, the zinc and nickel as sulphates. The results are shown in Table 1, each figure being a mean value for 20 plants in two replicates.

The populations are numbered 1–8 as follows: 1, A population of *A. alba* (identified as *A. stolonifera* L.) from a site at Piésky, Slovakia, described by Prát[2] as soil enriched in copper by a stream flowing from an old copper mine (provided by Prof. Fott and Dr. Jan Futák). 2, Population *D* of *A. stolonifera* from Port Meadow, Oxfordshire[3]. 3, *A. tenuis* from a copper-poisoned soil in an old mining area near Parys Mountain, Anglesey. 4, *A. tenuis* grown from seed provided by Dr. W. Krause, and described by him as being from the 'poison spot' of an old nickel mine in the Black Forest. 5, *A. tenuis* also provided by Dr. Krause, seed obtained from normal heath and

Table 1. Root Growth in Toxic Solution as a Percentage of Normal Growth

Concentration (micromoles/l.)		Population							
		1	2	3	4	5	6	7	8
Cu	5	72	4	83	66	7	4	34	4
	10	40	0	49	51	6	1	12	0
	15	20	0	32	29	1	0	4	0
Ni	5	27	12	50	103	43	60	50	30
	10	9	2	11	84	18	43	19	9
	15	4	0	3	70	4	14	10	0
Pb	75	26	18	37	27	16	22	80	28
	125	17	9	20	22	10	18	62	12
Zn	20	37	38	61	65	35	54	54	34
	40	21	17	33	42	19	38	48	21
	60	8	4	24	23	10	27	30	13

near the mine. 6, *A. tenuis* from a mine spoil-heap in North Wales, at first thought to contain lead, but now known to contain no significant amount of lead, but some zinc and possibly other metals. 7, *A. tenuis* from Goginan mine, Cardiganshire, previously described as resistant by Bradshaw[4]. The soil contains lead and zinc in toxic amounts. 8, *A. tenuis* from a meadow near Capel Bangor, Cardiganshire.

Each population except 2, 5 and 8, which were the controls, shows a marked degree of resistance to poisoning by the metals known to be present on the sites from which they were collected. The rather high degree of tolerance shown by the control 5 to nickel may be due to it having been grown in the same container as 4, which may have been removing a significant amount of nickel from the solution. However, without information as to how close to the mine the seed was taken, the possibility of some gene-flow from the nickel-resistant population cannot be eliminated.

A lesser degree of tolerance is also shown, in general, to other metals not known to be present. In the absence of detailed soil analyses, which are proceeding, the presence of other metals in the soil is possible, but it is suggested that the tolerance shown is due to an extension of the resistance mechanism operative against the main toxic agent. Population 6 is rather a special case, but seems to show its main resistance to nickel, although this is not so marked as in the Black Forest population.

On these sites the plant cover is generally very poor, especially where the calcium content is low, and very few species can cope with such an extreme environment. It is notable that two closely related species have proved capable of adapting themselves to meet these conditions with respect to a number of metals in several localities during the short time in which the sites have been available. The necessary genetic variation seems to be present in the genus *Agrostis* throughout a large part of its range.

The order of toxicity shown is copper > nickel > zinc > lead, which agrees with that given by Hewitt[5] and De Kock[6] for a range of plants, and also follows the Mellor–Malley series[7] for the order of stability of metallic-organic complexes. Furthermore, there are indications that the greater the degree of toxicity the more clear cut is the tolerance of it, excluding zinc, where no highly resistant population has yet been found. This lends support to the hypothesis that the resistance is due to the metal ions being bound internally by a process analogous to chelation; such a process would be most effective where the chelates are most stable. This would also explain the high concentrations of toxic metals in plants from poisonous sites noted by various authors[8,9].

However, unlike chelation the process is fairly specific for individual metals. Where activity is shown against lead, a lesser activity is shown against copper; not a higher as would be expected from the series quoted above. The advantage of this to the plant is apparent, in that it does not have micronutrient ions, which may not be abundant, made unavailable internally. If the plants have succeeded in elaborating a series of compounds capable of specific chelation, further study may be rewarding.

D. Jowett

Department of Agricultural Botany,
University College of North Wales,
Bangor.
July 16.

[1] Wilkins, D. A., *Nature*, **180**, 37 (1957).
[2] Prát, S., *Ber. Deutsch. Bot. Ges.*, **52**, 65 (1934).
[3] Bradshaw, A. D., *Nature*, **169**, 1098 (1952).
[4] Bradshaw, A. D., *New Phytol.*, **57**, 66 (1958).
[5] Hewitt, E. J., Long Ashton Res. Stat. Ann. Rep., 66 (1948).
[6] De Kock, P. C., *Ann. Bot.*, N.S., **20**, 133 (1957).
[7] Mellor, D. P., and Malley, L., *Nature*, **161**, 436 (1948).
[8] Rune, O., *Acta Phytogeog. Suec.*, **31**, 1 (1953).
[9] Spence, D. H. N., *J. Ecol.*, **45**, 917 (1957).

Fleas of the Rat in Tanganyika

The flea records presented here have been based on a two-year study of *Rattus rattus* (L.), the commensal rodent of Tanganyika. No previous list of the flea fauna of *R. rattus* of Tanganyika is known. In all 1,788 specimens of *R. rattus* were obtained and their fleas collected.

The term *Rattus rattus* (L.) used here is one of convenience and embraces the very mixed, interbred population formed of strains of *R. r. rattus*, *R. r. frugivorus*, *R. r. alexandrinus* and *R. r. wroughtoni*, that is peculiar to Tanganyika and perhaps to other parts of East Africa.

Table 1

Fleas	No. collected	Percentage of collection
Xenopsylla brasiliensis (Baker)	723	71·72
Xenopsylla cheopis (Rothschild)	220	21·72
Leptopsylla aethiopicus (Rothschild)	18	1·77
Ctenocephalides felis strongylus Jordan	16	1·58
Echidnophaga gallinaceus (Westwood)	12	1·18
Dinopsyllus longifrons lypusus J. and R.	7	0·69
Pulex irritans Linnaeus	6	0·59
Ctenophthalmus cabirus Jordan	6	0·59
	1,008	

The dominant species, *X. brasiliensis*, was distributed and was dominant in urban and rural areas from sea-level to 6,000 ft., whereas *X. cheopis* was not found above 4,000 ft. *P. irritans* and *Ct. f. strongylus* were fleas of urban rats, but *D. l. lypusus*, *L. aethiopicus* and *Ct. cabirus* were confined to rats from the rural areas. The cosmopolitan *E. gallinaceus* was found in both urban and rural areas.

My thanks are due to the Director of Medical Services, Tanganyika, for permission to publish this communication; and to Dr. B. de Meillon, of the South African Institute for Medical Research, and Mr. G. H. E. Hopkins, of the Zoological Museum, Tring, for identification of the fleas.

J. G. Halcrow

Medical Department,
P.O. Box 110,
Morogoro, Tanganyika.
July 1.

FORTHCOMING EVENTS

*(Meeting marked with an asterisk * is open to the public)*

Monday, September 22

Institution of Mining and Metallurgy (at the Royal Institution, 21 Albemarle Street, London, W.1), at 5.30 p.m.—Dr. Anton Gray: "The Future of Mineral Exploration" (Fourth Sir Julius Wernher Memorial Lecture).

Tuesday, September 23

Radar and Electronics Association (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7 p.m.—Mr. James Moir: "Stereophonic Sound Systems".

Tuesday, September 23—Wednesday, September 24

Institution of Mining and Metallurgy (in co-operation with the United Kingdom Metal Mining Association, at 21 Tothill Street, London, S.W.1), at 10 a.m. daily—Symposium on "The Future of Non-Ferrous Mining in Great Britain and Ireland".

Tuesday, September 23—Thursday, September 25

Institution of Mechanical Engineers (at 1 Birdcage Walk, London, S.W.1)—International Conference on "Gearing".

Wednesday, September 24

British Institution of Radio Engineers (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Mr. A. I. Godfrey: "Statistics in Radio and Television Manufacture".

Society of Chemical Industry, Plastics and Polymer Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. M. Szwarc (Syracuse University): "'Living' Polymers".

Oil and Colour Chemists' Association, London Section (joint meeting with the Society of Dyers and Colourists, at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Dr. J. L. Moilliet: "Surface Activity—the General Concept and some Consequences".

Wednesday, September 24—Friday, September 26

Institute of Petroleum Hydrocarbon Research Group—Mass Spectrometry Panel, and Committee E-14 of the American Society for Testing Materials in the Beveridge Hall, Senate House, University of London, Gower Street, London, W.C.1)—Conference on "Mass Spectrometry".

Wednesday, September 24—Friday, October 3

Industrial Fuel Efficiency Exhibition (at the National Hall, Olympia, London).*

Thursday, September 25, Friday, September 26, Tuesday, September 30 and Wednesday, October 1, from 10.30 a.m. to 12.30 p.m. daily—Series of Technical Meetings on "Lower Costs and Higher Production through Better Fuel Utilization".

Thursday, September 25—Friday, September 26

Institute of Biology (in the Lecture Hall, Royal Geographical Society, Kensington Gore, London, S.W.7), at 10 a.m. daily—Symposium on "The Effects of Pollution on Living Material".

Friday, September 26—Saturday, September 27

Institute of Physics, Electronics Group (at University College, London, W.C.1)—Meeting on "Solid State Memory and Switching Devices".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Resident Supervisor (with a degree in agriculture and a special interest in animal husbandry) for Certificate Courses in Agriculture—The Secretary, The North of Scotland College of Agriculture, 414 Union Street, Aberdeen (September 23).

Research Assistant (graduate in bacteriology) in Chemistry, to carry out routine growing of micro-organisms in connexion with a biosynthesis programme—The Registrar, The University, Manchester (September 29).

Senior Lecturer or Lecturer in Botany—The Registrar, The University, Manchester (September 30).

Lecturer (with a medical qualification) in Bacteriology at the Royal Infirmary, Glasgow—The Secretary of University Court, The University, Glasgow (October 10).

Lecturer (with an honours degree in mathematics or mathematical physics, lecturing experience, and preferably experience in some aeronautical subject and/or numerical methods) in the Department of Mathematics—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks (October 11).

Keeper of the Department of Geology—The Director, National Museum of Wales, Cardiff (October 24).

Chair of Solid State Physics tenable at the Imperial College of Science and Technology—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 27).

Lecturer in Physiology—The Secretary, The University, Aberdeen (October 31).

Lecturer in Psychology at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 31).

Lecturer or Assistant Lecturer in Biochemistry at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 31).

Lecturer/Senior Lecturer in Pathology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 31).

Lecturer (with a degree in science or agricultural science and interests in the fields of physical and inorganic chemistry) in Agricultural Chemistry (Physical and Inorganic) at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 31).

Chair of Electrical Engineering tenable at King's College, Newcastle-upon-Tyne—The Registrar, University Office, 46 North Bailey, Durham (November 15).

Lecturer in Physics, and a Senior Lecturer or Senior Research Fellow in Theoretical Physics at the University of New England, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, November 28).

Ralston Professor of Zoology at the University of Tasmania, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, December 15).

Agronomist at the West African Institute for Oil Palm Research, for research work on the cultivation of the oil palm; and Plant Physiologists (2), one to review present methods of production and initiate ways of increasing the supply of palm wine, and the other to carry out leaf analysis and other investigations into the nutrition of the oil palm—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.197/199/013.

Assistant Lecturer (Grade B) in Electrical Engineering, to assist in courses for O.N.C. and H.N.C. in electrical engineering; and an Assistant Lecturer (Grade B) in Applied Physics, for O.N.C. course developing to H.N.C. level in Session 1959–60—The Principal, Farnborough Technical College, Boundary Road, Farnborough, Hants.

Assistants (either sex, with a good reading knowledge of Russian or Italian, and preferably scientific interests), for abstracting papers in economic entomology—The Director, Commonwealth Institute of Entomology, 56 Queen's Gate, London, S.W.7.

Biochemist, basic grade (A.R.I.C. or B.Sc. with honours in chemistry or biochemistry)—The House Governor, Queen Elizabeth Hospital, Edgbaston, Birmingham 15.

Lecturer (with a good honours degree in chemistry or F.R.I.C.) in Inorganic Chemistry at Coventry Technical College—Director of Education, Council Offices, Coventry.

Research Assistant (with a good first degree in botany or horticulture, and preferably research experience and a higher degree), for work on the physiology of flowering in carnations—Prof. O. V. S. Heath, Department of Horticulture, The University, Reading.

Scientific Officer (with a first- or good second-class honours degree), to lead a section applying the techniques of gamma-ray spectrometry to the analysis of radioactive material and to expand the programme—Group Recruitment Officer (1193/34), United Kingdom Atomic Energy Authority, Atomic Energy Research Establishment, Harwell, Didcot, Berks.

Technical Research Assistant in the Physiology Department—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Guide to Evening Classes in Middlesex, Session 1958–59. Pp. 48+4 plates. (London: Middlesex County Council Education Committee, Education Offices, 1958.) [227

University Grants Committee. Returns from Universities and University Colleges in receipt of Treasury Grant, Academic Year 1956–1957. Pp. 50. (Cmnd. 477.) (London: H.M. Stationery Office, 1958.) 4s. net. [227

Biochemical Pharmacology, Vol. 1, No. 1 (July, 1958.) Pp. 104. Subscription (including postage): (A) £6 (U.S.A. 17 dollars) per volume; (B) for subscribers certifying that the journal is for their own personal use—£5 5s. (15 dollars) per annum. Four issues per volume. (London: Pergamon Press, 1958.) [227

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.,
ST. MARTIN'S STREET, LONDON, W.C.2.
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Annual subscription £7/15/–, payable in advance, postage paid to any part of the world

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office